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TABLE OF CONTENTS

Factors affecting the development of bacterial soft rot of potato tuber initials ..... R. S. DAVIDSON 673

The influence of cultural conditions on flag smut of wheat. A. F. EL-HELALY 688

Stem-end rot of strawberries. CONSTANTINE J. ALEXOPOULOS AND DONALD CATION 698

Two interesting leaf spots of fig ..... E. C. TIMS AND L. S. OLIVE 707

Physiologic specialization in Guignardia bidwelli, cause of black rot of Vitis and Parthenocissus species ..... E. S. LUTTRELL 716

Studies of two viruses causing mosaic diseases of soybeans. ROBERT A. CONOVER 724

Collar injury of apple trees associated with waterlogged soil. J. S. COOLEY 736

A study of the fungicidal action of 8-quinolinol and some of its derivatives ..... CURTIS L. MASON 740

Phytopathological Notes 752

New hosts for Botrytis elliptica ..... NEIL ALLAN MACLEAN

Phytophthora stem canker of sesame in Peru. BOWEN S. CRANDALL AND JAVIER DIEGUEZ C.

A technique for growing citrus seedlings under aseptic conditions ..... INAM U. KHAN

A method of testing beans for resistance to bacterial blights. C. F. ANDRUS

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FACTORS AFFECTING THE DEVELOPMENT OF BACTERIAL SOFT ROT OF POTATO TUBER INITIALS[1]

R. S. DAVIDSON[2]

(Accepted for publication April 1, 1948)

Potatoes grown in low, poorly drained areas produce low yields when excessive rainfall prevails for several days during the period of tuber initial formation. Lenticel infection of tuber initials, similar to that described by Smith and Ramsey (27) on early harvested mature tubers, was observed by the writer during field studies on the incidence of bacterial soft-rot at the Minnesota Agricultural Experiment Station. Bacterial lenticel infection of mature potato tubers, as observed at harvest, has been reported (13, 14, 15, 24, 25, and 26), and the pathological histology and identity of the bacteria involved have been studied (27). This paper deals with the environmental conditions under which lentical infection of tuber initials may occur, the extent of such infection, and the bacterial organisms involved. These studies were conducted at the Minnesota and the Rhode Island Agricultural Experiment Stations.

MATERIALS AND METHODS

At the Minnesota Station, Irish Cobbler potato plants were grown in 8-inch clay pots, in the greenhouse. At the Rhode Island Station, Irish Cobbler and Green Mountain plants were grown in Wagner-type subirrigation pots as described by Wheeler et al. (31) or plants were grown in greenhouse ground beds 48 by 60 inches and 18 inches deep with a gravel underlay.

Soils that had been under a cultivation program which included potatoes in the rotation were used. In Minnesota, sandy loam from University Farm was used; and Bridgehampton very fine sandy loam from the Experiment Station Farm, where potatoes had been grown continuously for at least 5 years, was used in Rhode Island.

In the Minnesota experiments, all fertilizer was applied as follows: 40 lb. N, 240 lb. P_2O_5, and 120 lb. K_2O per acre. The nitrogen was derived from sulphate of ammonia, the P_2O_5 from concentrated superphosphate (43 per cent P_2O_5), and the K_2O from muriate of potash. In the Rhode Island experiments, with the exception of specific studies on the relation between varying amounts of inorganic fertilizer constituents and the occurrence of infection, fertilizers were applied at the following rates per acre: 90 lb. N,

[1] Paper No. 2393, Scientific Journal Series, Minnesota Agricultural Experiment Station, and Contribution No. 704 of the Rhode Island Agricultural Experiment Station.

The results presented in this paper are based on investigations made at the Minnesota and Rhode Island Agricultural Experiment Stations.

Presented also to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, March, 1947.

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1/3 derived from nitrate of soda and 2/3 from sulfate of ammonia; 180 lb. P_2O_5, 5/9 of which was derived from superphosphate and 4/9 from concentrated superphosphate (47.5 per cent P_2O_5); 180 lb. K_2O, from muriate of potash. As a safeguard against magnesium deficiency, 30 lb. MgO as calcined magnesite (88.5 per cent MgO) was added.

It was evident from the outset that soil moistures produced in the greenhouse would not be entirely representative of field conditions. Nevertheless, an attempt was made to simulate field conditions in so far as possible. All water added to the soil was on the basis of monthly rainfall equivalent. To obtain a standard for pots and beds the amount of water necessary to approximate a given monthly rainfall rate was determined on the basis of the surface area of an individual pot and a greenhouse bed. Water was added at daily intervals, 0.4 gallon equalling one inch of rainfall for a greenhouse bed and 61 cc. equalling one inch for each Wagner pot. Water was added to the greenhouse beds from overhead and was measured by means of a disc water meter. Water was applied to the surface of the soil in the Wagner pots by direct measurement in cubic centimeters.

Soil moisture in the greenhouse beds and in the Wagner pots was determined on the basis of oven-dry weight, after water had been applied at several established rates to each. Triplicate soil samples for moisture determinations were taken, from a depth of approximately 4 inches, at least every other day, with a butter trier. In the beginning a series of soil moisture determinations was made for Bridgehampton very fine sandy loam; these data were used to compute the amount of water added to the soil in subsequent experiments. On the basis of oven-dry weight determinations, the water-holding capacity of the soil in the beds was approximately 29 per cent, while in the pots it varied between 32 and 35 per cent. Tap water was used throughout the investigations.

STUDIES ON INFECTION OF TUBER INITIALS

The Effect of Soil Temperature

A study was made of the effect of high soil moisture and various soil temperatures on bacterial infection of tuber initials of the Irish Cobbler variety. Constant temperature tanks, described by Christensen (5), were maintained at 4°, 18°, 24°, 27°, 34°, and 39° C.

Each tank accommodated 8 pots, one plant in a pot. Pots were suspended by wire racks attached below the collars of the pots so as to permit complete saturation of the soil with water from the temperature tank, but so as to avoid standing surface water. One potted plant from each of the 7 tanks was removed at approximately 12-hour intervals, the first series of 7 plants being examined 24 hours after the plants were placed in the tanks. The final series of plants was examined 36 hours after the previous series. Each plant was removed from the pot, washed in running tap water, and the number of healthy and infected tubers recorded (Table 1). The experiment was repeated using the same variety and the same soil temperatures. Plants

were examined at 48-hour intervals, beginning 24 hours after the soil was saturated. These data also are recorded in table 1.

Although this study was concerned principally with tuber initials, the response of the plants to environmental conditions also was observed. Blackleg symptoms, as described by Morse (22), appeared only in this phase of the work (Table 1).

No proliferation of lenticels was observed on tuber initials at 4° C. in less than 72 hours. About 60 hours after the beginning of the first experiment, the temperature in the 4° C. tank rose to 10° for about 8 hours because of insufficient ice. Although 4° C. is near the minimum for growth

TABLE 1.—*The relation of soil temperature to bacterial lenticel infection in tuber initials in flooded soils in two experiments*

Soil (°C.) temperature	Time flooded (hours)							
	24	31	47	56	72	80	96	118
	Percentage of tuber-initial infection							
4	0	0	0	0	50	33	.....	100
	0	.....	0	.....	0	.....	100	.....
16	0	0	0	0	0	25	.....	50
	0	.....	0	.....	0	.....	100	.....
21	0	0	83	37	50	100	.....	100[a]
	0	.....	62	.....	100	.....	100	.....
24	0	0	55	100	100	83	.....	100[a]
	25	.....	100	.....	100	.....	100	.....
27	0	0	100	100	100[a]	100[a]	.....	100[a]
	50	.....	100	.....	100	.....	100	.....
34	29	100	100	100[a]	100[a]	100[a]	.....	100[a]
	68	.....	100	.....	100	.....	100[a]	.....
39	100	100	100[a]	100[a]	100[a]	100[a]	.....	100[a]
	100	.....	100	.....	100	.....	100[a]	.....
Control	0	0	0	0	0	0	.....	0
	0	.....	0	.....	0	.....	0	.....

[a] Plants with symptoms of blackleg at time of examination.

of the bacterial organisms associated with soft rot, the higher temperature probably increased the bacterial activity in the soil and favored lenticel proliferation, since a slight amount of proliferation was observed in the initials examined after 72 hours. The combination of the higher temperature and lenticel proliferation probably favored entrance of the bacteria. When the original temperature was restored, periderm formation probably was retarded enough to prevent normal development and maturation of the periderm cells. Therefore, they were not resistant to the dissolving enzymes produced by the bacteria. The lenticels of potato tubers proliferate very little at low soil temperatures according to Devaux (8). He found that the optimum for proliferation was between 20° and 30° C. This was true also in the present study, where lenticel proliferation increased as the temperature increased within the 20° to 30° C. range.

The low percentages of infected tuber initials at 21° C. (Table 1) with

56 and 72 hours' exposure in moist soil may have been due to the complete disintegration of a portion of the infected initials, thus making an accurate count of total number of infected tubers impossible.

The Effect of Soil Fertility

An experiment was conducted to determine the effects of different ratios of nitrogen, phosphorus, and potassium on lenticel infection in saturated soils. The standard fertilizer treatment was a 6–12–12 grade. The following 6 modifications of this standard, as well as an unfertilized treatment, were included: 0–12–12, 6–0–12, 6–12–0, 12–12–12, 6–24–12, and 6–12–24.

Each fertilizer grade was applied to 24 individual Wagner pots; 12 were planted with a single seedpiece of Irish Cobbler variety and the remaining 12 with Green Mountain variety. The various fertilizer combinations were mixed thoroughly with the top 4 inches of soil in each pot before planting. Twenty-four pots, 12 of each variety, were prepared without fertilizer treatment. The planted pots were placed in the field during the last week in May with treatments randomized within the variety. The pots were protected from all natural rainfall. Throughout the early part of the growing period the soil moisture in all pots was maintained at 20 to 22 per cent on oven-dry basis. Seventy days following planting, the moisture content of the soil was increased to 33 to 35 per cent, the approximate field capacity of the soil, and was maintained for 168 hours. The plants were removed from 24 pots, 12 each of the 2 varieties, after 96, 120, 144, and

TABLE 2.—*The percentage of tuber initials of Irish Cobbler and Green Mountain varieties infected through the lenticels in soils saturated for different lengths of time and fertilized with different grades*

Variety and flooding time	Fertilizer grade applied at time of planting							
	0–0–0	0–12–12	6–0–12	6–12–0	6–12–12	12–12–12	6–24–12	6–12–24
	Infected tubers (per cent)							
Green Mountain								
Flooded 96 hrs.	48	30	63	52	39	66	52	50
120 hrs.	28	54	75	60	43	46	43	63
144 hrs.	68	44	67	51	62	61	65	60
168 hrs.	45	55	57	54	72	59	81	78
Average[a]	47	46	65	54	54	58	60	63
Irish Cobbler								
Flooded 96 hrs.	36	48	71	52	67	56	54	41
120 hrs.	64	52	62	63	45	65	51	34
144 hrs.	44	50	71	37	59	43	60	37
168 hrs.	53	41	75	57	55	83	65	53
Average[a]	49	48	70	52	56	62	58	41
Average both varieties[b]	48	47	68	53	55	60	59	52

[a] Difference necessary for significance at 5 per cent level is 15.6 per cent.
[b] Difference necessary for significance at 5 per cent level is 11.5 per cent.

168 hours. Roots and tuber initials were washed thoroughly in running tap water. The number of tuber initials per plant, the number of infected initials, the degree of lenticel proliferation, and the condition of the seed-piece were recorded.

The percentage of infected tuber initials for each plant was calculated and the data treated statistically according to the recommendations of Hayes and Immer (16). Before an analysis of variance was made, the actual percentages were converted to degrees, as suggested by Bliss (3). The differences in percentage of infected tubers in the different fertilizer treatments were significant at the one per cent level. There was no significant difference between varieties or between any of the first or second order interactions. The average percentages of tuber initials infected in 3 replicates of each treatment for each of the 2 varieties are in table 2.

The Effect of Atmospheric Humidity

Irish Cobbler and Green Mountain plants were grown in Wagner pots in the greenhouse at 18° C. The pots were weighed daily and soil moisture

TABLE 3.—*The percentage of tuber initials of Irish Cobbler and Green Mountain varieties infected at different soil moistures and relative humidities of the air*

Variety	Percentage soil moisture on dry weight basis	Percentage relative humidity of atmosphere	Percentage of tuber initials infected after the number of hours indicated					
			48	95	120	144	168	192
Cobbler	35	85	0	0	25	23	29	55
	35	30	0	0	0	0	0	0
	22	85	0	0	0	0	0	0
	22	30	0	0	0	0	0	0
Green Mountain	35	85	0	50	20	60	45	25
	35	30	0	0	0	0	0	0
	22	85	0	0	0	0	0	0
	22	30	0	0	0	0	0	0

maintained at 22 per cent. Fifty-two days after planting, the soil moisture in half of the pots of each variety was increased to 35 per cent. One half of the pots of each variety at each of the two moisture levels was placed in a Wardian chamber. Two-inch metal standards for the pots kept them above the water that filled the metal pan to a depth of one inch. The relative humidity of the atmosphere in the chamber was about 85 per cent. The remaining lots of potted plants were kept in the greenhouse at a relative humidity of approximately 30 per cent. Daily greenhouse temperatures varied between 19° and 21° C. throughout the 192-hour period. Individual plants from the 4 groups of each variety were examined at 24- to 48-hour intervals, beginning 48 hours after the increased soil moisture and high relative humidity were provided. The results, in table 3, are based on the percentage of infected tuber initials present under the stated conditions of soil moisture and atmospheric humidity. Lenticel infection of tuber initials of

both varieties was found only on plants grown in saturated soil and in an atmosphere with a relative humidity of 85 per cent.

Relation Between Soil Moisture and Lenticel Behavior

According to Hayward (17), lenticels are formed by a division of the cortical cells which produces a group of loosely organized cells, termed complementary cells. Pressure on the complementary cells, resulting from development and growth of the periderm, causes a rupture of the epidermis at the original stomatal opening. Lenticel formation usually results from a local cellular proliferation with the formation of a channel or opening, according to Devaux (9). He also states that a lenticel is an organ in the process of continual growth and destruction. In the potato tuber, the process of continual growth and destruction maintains a state of equilibrium when soil moisture conditions are optimum for potato plant growth. This natural balance between growth and destruction is accompanied by suberization. In wet soils, the rate of growth exceeds the rate of destruction and suberization of the complementary cells, thus creating openings through which soil-borne microorganisms enter.

It is common knowledge that lenticels swell and proliferate when potato tubers are grown in, or exposed to, high soil moistures. De Bary (7) demonstrated that cells formed by the meristematic layer of cells of the lenticels have "hygroscopicity," a tendency to take up water and become swollen. He showed that much of the conspicuous extrusion of the complementary cells which characterizes proliferated lenticels resulted from the swelling of these cells. He also observed that very little proliferation of lenticels occurred at low temperatures and determined the optimum for proliferation to be 20° to 30° C. According to Olufsen (23), moderate soil moistures are the most essential requirement for periderm formation, while excessive soil moistures hinder periderm formation and cause cell proliferation.

Most of the lenticels on the surface of tuber initials were in the apical quarter or third of the tuber. On mature tubers the lenticels were more evenly distributed, with a slight preponderance at the extreme apical end. Lenticel distribution on immature tubers was not always apparent to the naked eye because lenticels were very small and inconspicuous in the apical region. Stomata were found in various stages of transformation to lenticels, progressing from the apical end toward the stem end of the tuber. Developing lenticels were much smaller, less swollen, and less proliferated in the apical region than near the stem end. The extent of the proliferation appeared to vary directly with increasing amounts of soil moisture.

In the present study the bacteria entered the tuber initials through the lenticels in all instances, so far as could be determined. On tuber initials in soil saturated to field capacity, infection is likely to be most abundant near the apical end. The first signs of lenticel infection were most frequent in the apical third of the tuber initial, although lenticels near the stem end

occasionally became infected first. When lenticels in the stem end of the tuber became infected first, the progress of the infection was slow and did not become so extensive as that in the apical region.

Infections through lenticels resulted in very uniform, circular, water-soaked areas from 1 mm. to as much as 20 mm. in diameter. The periphery was usually distinct and uniform. On freshly dug tuber initials the infected spots appear water-soaked and sometimes slightly brown. After exposure to the air for an hour or more, the infected areas become deep pink, and after 24 to 48 hours' exposure to the air, they become brown with a sunken pox-effect on the surface.

The depth of the infection appeared to depend on the age of the tuber initial and age of the infection. In general, the depth was governed by the thickness of the cortex of the tuber initial. The cortex is proportionately thicker in initials than in mature tubers. On tuber initials 15 to 20 mm. in diameter, the depth of the infected area is about equal to the radius of the infected area on the surface. When the infected area on the surface was 10 to 12 mm. in diameter, the depth of the infected areas extended beyond the cortical region. Infection spots often coalesced to form large surface spots, with rather deep penetration into the tuber tissues.

The epidermis covering a single infected lenticel was often distended, forming a blister over the infected area. When freshly dug tubers with blisters were submerged in water, gas bubbles issued from the lenticels. A cracking sound, as the bursting of a small balloon, was detected when pressure was applied to the blisters. Occasionally the entire contents of the tuber decayed, the epidermis merely providing a bag for the watery contents. Once a lenticel became infected, proliferation ceased.

The infected cortex was watery to viscous. The epidermis over infected lenticals was broken easily and often could be washed away with running tap water. The decayed tissue beneath the epidermis was readily washed away also, leaving a uniform crater-like pit. When the epidermis covering several adjacent infected lenticels was removed, the exposed tissue appeared as if chewed by a rodent.

The odor of the decayed tissue was variable. Some infected lenticels, when carefully lanced with a scalpel, were odorless; others had a very strong sour odor, especially those in advanced stages of decay and those resulting from coalescence of several infections. Secondary putrifying organisms may be responsible for the odor in many instances.

Bacteria were consistently isolated from infected lenticels. Several hundred isolations were made, but fungi were never obtained. Actinomyces appeared in some dilution plates but never without bacteria.

Infected lenticels were observed occasionally on the stolons. The only instance in which affected stems were found was during the studies of the effect of various soil temperatures (Table 1). There were many infected initials on plants with blackleg symptoms; however, no association between these two pathologic responses of the host was established in the present study.

TABLE 4.—*Physiological characteristics of the cultural types within four species of bacteria isolated from infected lenticels and other parts of potato plants*

Cultural types[a, b]	Physiological tests[c]														
	Gelatin	Starch	M. R.	V. P.	Nitrate	H_2S	Xylose	Dextrose	Mannose	Sucrose	Lactose	Maltose	Glycerol	Salicin	Aesculin
Erwinia carotovora															
1	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	−
2	−	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	AG
3	−	−	+	+	+	+	AG	AG	AG	AG	AG	AG	AG	AG	AG
4	−	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	−
5	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	a	AG	AG
6	−	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	ab
7	−	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	−
8	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	AG	AG	AG
9	−	−	+	−	+	+	AG	AG	AG	AG	AG	AG	AG	−	−
10	+	+	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	AG
11	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	AG
12	−	−	+	−	+	−	AG	AG	AG	AG	Ag	AG	A	AG	AG
13	−	−	−	+	+	+	AG	AG	AG	AG	AG	AG	AG	AG	AG
14	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	a	AG	AG
15	+	−	−	+	+	−	AG	AG	AG	AG	Ag	AG	A	AG	−
16	−	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
17	+	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
18	−	−	−	+	+	−	AG	AG	AG	AG	AG	AG	A	AG	a
19	+	−	−	+	+	−	AG	AG	AG	AG	AG	AG	AG	AG	A
20	+	+	−	+	+	−	AG	AG	AG	AG	A	AG	a	AG	A
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E. aroideae															
24	+	−	+	+	+	−	a	A	A	A	A	A	A	−	−
25	+	−	−	−	−	−	a	−	a	−	−	−	−	−	−
26	+	−	−	−	+	−	a	a	a	−	−	−	a	a	a
27	−	−	−	−	−	−	a	a	a	a	−	a	a	a	a
28	+	−	−	−	+g	−	a	A	a	−	a	−	a	−	−
29	−	+	−	−	−	−	a	A	A	a	a	a	−	*a*	*a*
30	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−
31	−	−	−	−	−	−	A	A	a	−	*a*	−	a	a	a
32[d]	+	−	+	−	+	−	A	A	a	A	A	−	A	A	a
Bacillus mesentericus															
33	+	+	+	+	+	−	a	A	A	a	a	A	a	A	*a*
34	+	+	+	+	+	−	a	A	A	a	a	A	a	A	a
35	+	+	+	+	+	−	a	A	A	a	a	A	a	A	A
36[d]	+	+	−	+	+	−	a	A	A	A	a	a	a	A	A
B. polymyxa															
37	+	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
38	−	−	−	+	+	−	AG	AG	AG	AG	Ab	AG	A	AG	AG
39	+	+	−	+	+	−	AG	AG	AG	AG	AG	AG	AG	AG	Ag
E. solanisapra															
40[d]	+	−	−	+	+	−	A	Ab	A	Ab	A	A	A	A	A
E. phytophthora															
41[d]	+	−	+	−	+	−	a	A	A	A	A	−	A	A	*a*
Escherichia coli															
42[d]	−	−	+	−	+	−	AG	AG	AG	AG	AG	AG	AG	AG	AG

[a] Cultures 1 to 32 and 40 to 42, inclusive, were Gram-negative; 33 to 39, inclusive, were Gram-positive and spore-formers. All isolates produced short rods and were aerobic

BACTERIAL IDENTIFICATION STUDIES

All microorganisms studied were bacteria isolated from infected lenticels on tuber initials and from stems of plants with blackleg symptoms. Single-colony isolations from cultures purified by the dilution-plate method were used in all the identifications and pathogenicity studies reported here. All bacterial isolates and type cultures were maintained on Bacto-nutrient agar or Bacto-nutrient broth. Identification tests were made according to the procedures outlined for pure-culture studies by the Society of American Bacteriologists (6). All bacterial isolates included in the identification studies were first tested for their ability to produce a soft-rot on potato slices in the laboratory. Only those isolates which rotted potato slices were included in the identification studies. The characteristics determined for the various isolates are summarized in table 4.

Gram stain reaction of all isolates was determined by the Hucker modification of the Gram stain. Standard culture 36 was selected as a check for Gram-positive reaction and standard culture 21 for Gram-negative reaction. All isolates were Gram-negative with the exception of 33, 34, 35, 37, 37a, and 38. The Gram-stain reaction was determined on smears prepared from 48-hour Bacto-nutrient agar-slant cultures.

All isolates were stained by the Schaeffer and Fulton modification of the Wirtz method of spore staining. Again, the two cultures used as standards in the Gram-stain reaction were employed as checks. The smears for the spore stains were prepared from 10-day-old Bacto-nutrient agar-slant cultures. Isolates 33, 34, 35, 37, 37a, and 38 were the only ones that produced spores.

Motility was determined in hanging drops prepared from 8-hour Bacto-nutrient broth cultures. All isolates were actively motile except standard culture 42.

Physiological Characteristics

Starch Hydrolysis. The ability of each isolate to hydrolyze starch was determined by means of the starch-agar plate technic. A loop transfer from a broth culture of each isolate was streaked on a plate of Bacto-nutrient

and facultatively anaerobic. All cultures except 42 were actively motile, negative for indole production, and pathogenic on potato slices.

[b] Each of these types was isolated once, except 1 to 3, inclusive, each of which was isolated three times; 4 to 7, inclusive, and 37 were isolated twice. Isolates identical in reaction to those listed are not included in the table but are referred to in the text as a and b.

[c] +, Positive for gelatin liquefaction, starch hydrolysis, Methyl Red Test, Voges-Proskauer Test, nitrate reduction, and H_2S production. −, Negative reaction for the above five tests and no visible change in color indicator or gas produced. *a*, Slight acid production, light pink color. a, Weak acid production, pink to orange color. A, Strong acid production, yellow to clear color. b, 1 to 5 per cent gas by volume in fermentation tube. g, 5 to 10 per cent gas by volume in fermentation tube. G, 10 to 100 per cent gas by volume in fermentation tube.

[d] Standard cultures 21 and 22 were obtained from Dr. W. H. Burkholder, Cornell University, who considers them typical of *E. carotovora;* 23, 32, 40 and 41 were obtained from the American Type Culture Collection; 39 was obtained from Dr. G. A. Ledingham, Division of Applied Biology, Ottawa, Canada; 36 and 42 from the Department of Bacteriology, University of Minnesota.

agar containing 0.2 per cent soluble starch. All plates were incubated at 26° to 28° C. for 48 hours, after which each plate was flooded with a saturated solution of iodine in 50 per cent ethyl alcohol. Isolates 10, 20, 29, 30, 33, 34, and 35 hydrolyzed starch; the others did not.

Fermentation of Sugars, Alcohols, and Glucosides. All isolates were tested for ability to ferment xylose, dextrose, mannose, sucrose, lactose, and maltose. Glycerol was used to determine the ability of the isolates to ferment an alcohol. Salicin and aesculin were selected to represent the glucosides. Bacto-phenol-red nutrient broth base containing 1 per cent of each of these nine compounds was used for all liquid media. All media for fermentation tests were prepared in Durham tubes, in 5 cc. aliquots, and sterilized for 20 min. at 121° C., except the media containing xylose, which was sterilized with flowing steam for 20 min. on 3 successive days.

The various media were inoculated with a single-loop transfer of a 24-hour broth culture. Each isolate and standard culture was tested in duplicate on each of the 9 media. All cultures for fermentation studies and other physiological tests were incubated at 28° C. The inoculated broths were examined after 24 hours and a daily check was made of each test for 2 weeks. The maximum amount of acid or gas, or both, produced during the 2-week period was taken as the final reading for each culture.

Liquefaction of Gelatin. Liquefaction of gelatin was determined on stab cultures in Bacto-nutrient gelatin incubated at 20° C. Of the 44 isolates studied, 15 liquefied gelatin. The cultures were kept for 28 days; however, all isolates which liquefied the medium did so in 4 to 9 days after inoculation. The type of liquefaction varied from stratiform to infundibuliform, with complete liquefaction in all positive cultures after 28 days. Details are given in table 4.

Reduction of Nitrates. Reduction of nitrates was determined by the sulphanilic acid alpha-naphthylamine test. All isolates with the exception of 25, 27, 29, and 31 reduced nitrates to nitrites. Only one isolate, 28, reduced nitrate with the evolution of gas. Gas production was determined for all cultures in Durham fermentation tubes.

Indole Production. The isolates were tested for the production of indole by the Ehrlich-Bohme method. Duplicate cultures of each isolate were inoculated into Bacto-tryptone broth media and incubated at 28° C. One culture of each isolate was tested for indole production after incubation for 24 hours, and the duplicate, after 48 hours. None of the isolates produced indole. A culture of *Escherichia coli*, 42, that was known to produce indole was included as a check. It gave a positive reaction after 24 and 48 hours.

Production of Hydrogen Sulphide. Hydrogen sulphide production is detected in bacterial cultures by the blackening produced when bacteria are grown in the presence of a metal such as lead, lead sulphide being produced. Only isolates 3, 3a, 3b, 9, and 13 were positive for hydrogen sulphide production.

Methyl Red Test. The methyl red test is used to detect high acidity

produced by certain organisms in the presence of dextrose. This test has been included in comparative studies of soft-rot bacteria by Stanley (29), and Dowson (10, 11). Bacto-M.R.-V.P. medium was used in the methyl red and "Voges-Proskauer Reaction." Of the isolates tested, 3, 3a, 3b, 9, 12, 24, 33, 34, and 35 gave a positive reaction.

"Voges-Proskauer Reaction." This test was made with the same medium as that for the methyl red test. The reactions of the various isolates to this test are in table 4.

As a result of the identification studies four species of bacteria were found associated with lenticel infection; *Erwinia carotovora* (Jones) Holland, *Erwinia aroideae* (Townsend) Holland, *Bacillus mesentericus* Trevisan, and *Bacillus polymyxa* (Prazmowski) Migula. Isolates 1 to 20, inclusive, are believed to be most similar to *Erwinia carotovora* as typified in the reactions of standard cultures 21, 22, and 23. Variation in the physiological tests was recorded; however, it was no greater than that recorded in standard cultures 21, 22, 23, 40, and 41. The variation in the physiological reactions of members of the *E. carotovora* group has been studied by Leach (20). The variation among the isolates here considered to be most similar to *E. carotovora* was no greater than that given by Leach (20) for the group, and that reported by Stanley (28).

Only isolates 10, 15, 17, 19, and 20 liquefied gelatin. If Leach's (20) grouping, which includes *Erwinia solanisapra* (Harrison) Holland, is followed, the variablility in gelatin liquefaction is confirmed. Bergey (2) records surface liquefaction to a depth of 2 mm. after 25 days' incubation for *E. solanisapra.* Where such slight amounts of liquefaction were observed in the present studies, negative reactions were recorded.

Standard culture 41 had a very weak acid reaction in aesculin broth, while all other standard cultures and literature listings for the test show acid production without gas. Those isolates classified here in the *Erwinia carotovora* group gave either a negative aesculin reaction, or both acid and gas positive reaction.

Isolate 9 gave a negative reaction in salicin broth, while all others produced acid and gas. Isolates 3, 8, 9, 13, and 19 produced both acid and gas in glycerol.

In 1904, Townsend (30) described a bacterial soft-rot of calla lily and designated *Erwinia aroideae* as the causal organism. Massey (21) and Wingard (32) reported a soft-rot of tomato fruits caused by *E. aroideae.* Massey (21) concluded from comparative tests of *E. aroideae* and *E. carotovora* that these two organisms, although closely related, should be maintained as separate species. Brierley (4) also included *E. aroideae* in comparative studies of bacteria which caused a rotting of potato tuber tissue under laboratory conditions. He stated, *"Erwinia aroideae* may be found destructive under field conditions also, when a survey of soft rots in the field is undertaken." Stanley (29) and Dowson (10, 11) suggested this organism be classified separately because of its consistent inability to produce gas in the various carbohydrate-containing broth media.

Isolates 24 to 31, inclusive, are believed to be most closely related to *Erwinia aroideae*. The reactions of these 8 isolates to the various tests are recorded in table 4. It is apparent that there is considerable deviation from the standard culture 32. Bergey (2) and Dowson (10, 11) recorded gelatin liquefaction for *E. aroideae*, but isolates 27, 29, and 31 did not liquefy it. Seven of the 8 isolates were negative for both the methyl red and "Voges-Proskauer Reaction," while isolate 24 was positive for both. Four of the 8 isolates apparently had lost the ability to reduce nitrates, while one, 28, reduced nitrates with the production of gas. None of the isolates produced hydrogen sulphide. Bergey (2) has recorded a positive reaction for this test, while a negative test was recorded for the standard culture 32 in these studies. Again, as in comparative studies of the isolates determined to be *E. carotovora*, considerable variation was found among the 8 isolates here assigned to the *E. aroideae* group. It seems most logical to classify the isolates in this group on the basis of their ability to rot potato tubers and their consistent production of acid without gas. Isolate 30 is included in the *E. aroideae* group because it causes rot, and may have produced acid, although in quantities too small to be detected with phenol-red indicator.

Brierley (4) isolated *Bacillus mesentericus* from a wound rot of potato tubers. Subsequently it rotted healthy potato tubers at temperatures of 20° C. and above in the laboratory. Some spore-forming bacteria of the *Bacillus subtilis* and *Bacillus polymyxa* types can dissolve the middle lamella of parenchymatous tissues (1).

The characters of the 3 isolates of Gram-positive, spore-forming bacilli, 33, 34, and 35, agree with those of standard culture 36 in all tests except the methyl red test, which was positive for all 3 isolates but negative for 36. Isolate 34 appeared as a variant of a culture of isolate 33 and rotted potatoes faster than its parent.

Dowson (12), isolated *Bacillus polymyxa* from stored potato tubers. More recently, Jackson and Henry (18) isolated the same spore-forming bacterium from potato soils in Alberta, Canada, and demonstrated that it could enter through wounds and rot potato tubers at high temperature and humidity.

Three isolates of Gram-positive, spore-forming bacilli, 37, 37a, and 38, gave typical *Bacillus polymyxa* reactions in the various tests. They did not hydrolyze starch, and 38 did not liquefy gelatin. Isolates 37a and 38 were obtained from infected lenticels. Isolate 37 was derived from the base of a potato stem with typical symptoms of blackleg.

DISCUSSION

Potato tuber initials grown in wet soils become infected with several species of bacteria that enter through proliferated lenticels. The higher the soil moisture, the greater was the lenticel proliferation and the number and extent of lenticel infections.

Lenticels are formed on tuber initials early in the development of the

initial. Lenticels near the stem end of the tuber initial may be mature while stomata are still present at the apex of the tuber. Infections occurred most frequently in the stem end two-thirds of the initial, with no infections in the extreme apical region. This observation suggests that lenticels more frequently than stomata are predisposed to infection in wet soils. Those lenticels in the middle third of the initial were more frequently infected than those in the extreme stem end, which further suggests that immature lenticels are more susceptible to infection than mature ones.

In proliferated lenticels, the complementary cells are formed rapidly, are thin walled, non-orderly in arrangement, and have large intercellular spaces. The cork layer, which normally forms beneath the complementary cells, is not present in actively proliferating lenticels in wet soils. Therefore, proliferated lenticels are not protected against entrance of pathogens such as soft-rot bacteria.

The amount of oxygen in wet soils may not be sufficient for suberization of the complementary cells and for cork formation; consequently, proliferated lenticels are further predisposed to infection, as Leach (19) has suggested in the case of blackleg.

Potato tuber initials grown in wet soils where high nitrogen fertilizers had been applied were very susceptible to lenticel infection, probably because of increased rate of complementary cell production, which thus provided less protection to the lenticel.

That the warm, muggy weather that often prevails when soils are wet is conducive to lenticel infection is suggested by the observation that infection of tuber initials was more severe on plants grown in atmospheres with a high relative humidity. The soil temperatures usually encountered under such circumstances would also be favorable to lenticel infection.

The soil environmental conditions in which lenticel infection occurs are also optimum for the growth of the 4 species of bacteria: *Erwinia carotovora, Erwinia aroideae, Bacillus mesentericus,* and *Bacillus polymyxa,* which are all facultative anaerobes and are known to be more active under partially anaerobic conditions. The anaerobic atmosphere of the rhizosphere was not conducive to the growth of fungi and, in extremely wet soils, prevented the development of Actinomyces. This unnatural balance in the soil microflora may reduce the prevalence of microorganisms that are antibiotic to the soft-rot bacteria. The increased bacterial activity also may be due to a symbiotic relation of the soft-rot bacteria and other bacteria which would not be present in soils of lower moisture content.

SUMMARY

1. Potato tuber initials can be completely rotted at very early stages in their development, especially in wet soils, by at least 4 species of bacteria: *Erwinia carotovora, Erwinia aroideae, Bacillus mesentericus,* and *Bacillus polymyxa.*

2. The rotting of the initials in wet soils was occasionally so extensive as to suggest that rotting may, in some instances, be responsible for low yields of potatoes.

3. In studies made to determine factors which predispose tuber initials to rot, two soil types were used; sandy loam from the Minnesota Agricultural Experiment Station Farm, St. Paul, and Bridgehampton very fine sandy loam from the Rhode Island Agricultural Experiment Station Farm, Kingston. Rotting was most abundant in both soils when they were saturated to field capacity.

4. Of a total of 30 isolates obtained from infected lenticels on potato tuber initials and potato plant stems grown in University Farm sandy loam, 26 were *Erwinia carotovora* and 3 were *Bacillus polymyxa*. From tuber initials grown in Bridgehampton very fine sandy loam, *Erwinia aroideae* was isolated 8 times, *Erwina carotovora* 4 times, and *Bacillus mesentericus* 3 times. This suggests that the relative prevalence of the soft-rot bacteria may vary in different regions or soils.

5. The development of lenticel infection is retarded by soil temperatures of 18° C. and lower and is accelerated at higher temperatures. All tuber initials on plants grown in saturated soils at 39° C. became infected within 24 hours, whereas the initials on plants grown at 4° and 18° C. required 96 hours for infection.

6. Lenticel infections were more abundant on tuber initials of the Irish Cobbler than on those of the Green Mountain variety in all experiments, but the differences were not statistically significant. The percentage of proliferated lenticels and the extent of proliferation on individual tubers were greater on initials of Cobblers than Green Mountains. There were more lenticels on Cobbler than on Green Mountain tuber initials.

7. In the present study, bacterial infection of tuber initials occurred only through lenticels.

8. Blackleg symptoms were observed on plants grown in saturated soil at temperatures of 21° C. and above, with the optimum for the disease symptoms between 34° and 39° C.

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THE INFLUENCE OF CULTURAL CONDITIONS ON FLAG SMUT OF WHEAT

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Flag smut, *Urocystis tritici* Koern., causes an average loss of less than 5 per cent of the wheat crop in Egypt, but under certain conditions it may cause a loss of 50 per cent or more. It is the object of this work to determine the factors which influence the spread and the distribution of the disease in Egypt and to find a cheap method of control.

The disease probably has been present in Egypt for a long time. It attacks the Hindi varieties of wheat, *Triticum vulgare*, which are known to have been grown in Egypt for hundreds of years. The pathogen may also have been introduced to this country with imported Australian grain during the Great War of 1914.

The first record of the disease in Egypt was made by Fahmy (See 7) in 1923. Melchers and others (7) found flag smut at Mansoura in 1928. Jones (5) reported the disease and stated "Ordinarily this disease is very rare, but it appears to be becoming more common and may demand treatment in some cases." Jones and Seif el-Nasr (6) stated that the disease was at its maximum near the center of the Delta: the highest figure of infection ever recorded in a field was 58 per cent, but the average percentage in a normal year was very much less.

It is most severe in lower Egypt (the Delta) and decreases gradually southwards, becoming rare in Upper Egypt. It has been reported in Sinai, but has not yet been seen in the Oases. The intensity of the disease varies from year to year.

METHOD AND MATERIAL

The Hindi D variety of wheat which is susceptible to flag smut disease, was used unless otherwise indicated. The seed was obtained from the Propagation Section of the Ministry of Agriculture. Spores for infection purposes were taken from diseased plants collected especially for this purpose. In infesting the experimental plots, a sample of light soil was infested by mixing it thoroughly with diseased straw and with spores at approximately the rate of 5 gm. of straw and 1 gm. of spores to 1 kilogram of soil. Each experimental plot received, at the proper time, approximately 1 kilogram of this inoculum, which was evenly spread on the surface of the soil and mixed with it to the depth required.

The artificially infested seed was prepared by adding approximately 0.5 gm. spores to 1 kilogram of seed, and then thoroughly mixing them.

The plants of each experiment were kept under observation from the time of sowing to the time of harvest. Readings were taken every fortnight and the total number of the smutted plants per plot were, then, recorded.

The wheat plants of many different plots, each plot being 2×5 meters, were counted and the average number per plot was 750 plants.

This work was carried out in the Field Stations of the Ministry of Agriculture at Dokki and of the Faculty of Agriculture of Farouk 1 University at Damanhour, and in the laboratories of the Plant Pathology Section of the said Ministry during 1940–1941, 1941–1942, and 1942–1943.

FACTORS INFLUENCING DISTRIBUTION OF DISEASE

The spread of the disease in Egypt depends on different cultural and environmental factors which act either together or separately. The main factors which influence the distribution and spread of the disease are: (1) date of sowing; (2) method of sowing; (3) manurial treatment; (4) smut spore load on the seed; (5) number of waterings.

Date of Sowing

Date of sowing plays an important part in the incidence of the disease. Tisdale, Dungan, and Leighty (14) reported that late sowing (after November 1), in Illinois, greatly reduced infection by flag smut. Millikan and Simo (11), in Australia, found that early sowing favored infection. Two experiments were designed in 1940–1941 and in 1941–1942 at Dokki to investigate this point. Artificially infested seed was sown on the following dates: Oct. 1, Oct. 15, Nov. 1, Nov. 15, Dec. 1, and December 15, 1940 and 1941, in artificially infested plots. Each treatment was reported six times. In figure 1, are presented the data obtained and the maximum and the minimum daily air temperatures for each period.

The effect of date of sowing on the incidence of the disease is very pronounced. The plants of the early sowings were almost free from the disease, while those of the late sowings, particularly in 1941, were very badly attacked. The highest infection occurred in the plants of the sowings of Dec. 1. The curves indicate that temperature appears to be the main factor in this case.

Method of Sowing

In Egypt, wheat is sown either by the "Afir" or by the "Herati" method. In the "Afir" method, sowing takes place on dry soil which is irrigated at once, in the "Herati" method sowing occurs on moderately moist soil which is, then, left without irrigation for about one month. These two methods of sowing influence the incidence of certain smut diseases. El-Helaly (2) working on kernel smut of sorghum stated that "Herati" sowing favors considerably the spread of the disease. Jones and Seif el-Nasr (6) working on smut diseases in Egypt found that the crops planted by the "Herati" method consistently bore more flag smut than the "Afir" planting.

In our experimental plots at Dokki, the Herati method of sowing favored infection. In 1940–1941 flag smut infection was 4 to 5 per cent in the four Afir sowings and ranged from 9 to 12 per cent in the four Herati sowings.

The next year, 1941–1942, the smut ranged from 2 to 10 per cent in Afir sowings and from 15 to 34 per cent in the Herati sowings. It seems that the underlying factors in this case may include one or more of the following: water drainage, soil temperature, depth of sowing, and soil moisture.

Drainage of Water of Irrigation at Sowing. Watering which follows sowing in the "Afir" method may remove, during drainage, the spores adhering to the seed coat, leaving the seed almost clean. El-Helaly (2) working on kernel smut of sorghum found that "the soil particles retain the smooth spores and, therefore, do not allow their passage through during

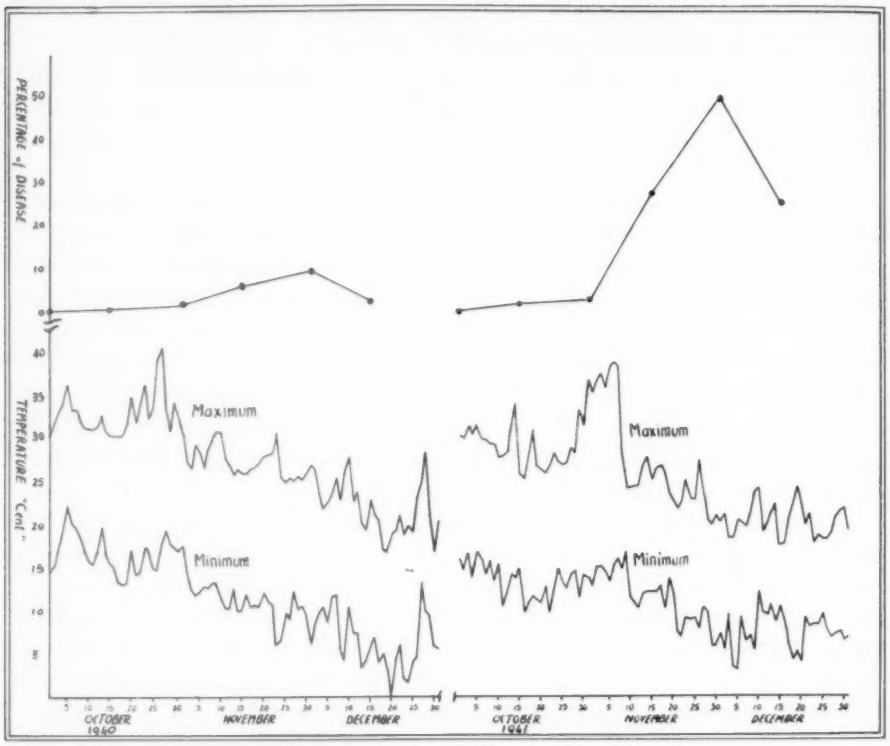

FIG. 1. The percentages of flag smut in plots of Hindi D wheat planted on different dates and the temperatures during the planting season at Dokki, Egypt, in 1940–1941 and 1941–1942.

irrigation." This may be true of the sculptured flag smut spores which may be clustered in spore balls.

Soil Temperature. Soil temperature in the "Herati" method may be more favorable for fungus attack. Jones and Seif el-Nasr (6) stated that the "Afir" plots were a fraction of a degree to two degrees C. cooler than the corresponding "Herati" plots.

Depth of Sowing. In the "Herati" method the seed is broadcast on wet soil and is then ploughed in, giving an average depth of about 8 cms.; in the "Afir" method the seed is broadcast on dry soil and is then covered by means of the zahhafa, a long narrow flat block of wood dragged

over the soil; by this method the seed becomes buried to a depth of about 4 cms. Miller and Millikan (8), Millikan and Simo (11) and Jones and Seif el-Nasr (6) reported that deep sowing favored infection by *Urocystis tritici*.

In order to ascertain the effect of depth of sowing on the incidence of flag smut disease, experiments were conducted in 1942–1943 at Damanhour using 3×7 meters, artificially infested plots. The plots were drilled with artificially infested wheat grain in strips at a depth of 0.5 cm., 2.5 cms., 5 cms., 7.5 cms., 10 cms., 15 cms., and 20 cms.

The "Herati" method was followed and great care was taken in order that the depths might be as accurate as possible. Table 1 contains the data obtained from these plots. The data agree with those of the earlier workers, *i.e.*, the disease increases with the increase in the depth of sowing.

TABLE 1.—*The stand of plants and the percentages of flag smut on three varieties of wheat sown at different depths at Damanhour in 1942–1943*

Depth of sowing, in cm.	Stand[a] and percentage[b] of flag smut in					
	Hindi D		Mabrouk		Hindi 62	
	Stand	Smut	Stand	Smut	Stand	Smut
0.5	15	9	9	8	11	5
2.5	41	28	36	30	42	28
5.0	41	38	37	43	40	33
7.5	42	44	39	57	40	37
10.0	41	44	40	50	40	33
15.0	41	50	33	54	36	41
20.0	37	52	30	66	31	55

[a] The number of plants in a plot, each number being the average of six.
[b] Each percentage is the average for six plots.

It is known that infection occurs when the living fungus comes in contact with recently germinated seedlings. The occurrence of infection is limited to the period between the time of germination of the seed and the appearance of the seedling above ground. In deep sowings the coleoptiles remain longer below the surface, growing in damp soil which will, also, keep the infecting fungus alive for a longer time. This condition will obviously lengthen the period of susceptibility and will, thus, increase the chances of infection.

Soil Moisture at Sowing. Soil moisture varies according to the two methods of sowing. With the "Herati" method, the soil is irrigated 8 to 15 days prior to sowing and is left until it is just moist enough for plowing. Any smut spores that have had soil moisture available for 8–15 days and a natural pre-soaking may put them in condition for germination and infection when the wheat seeds are sown. With the "Afir" method the soil usually has dried out for 40–50 days prior to sowing and water is first added at sowing time. There probably is insufficient soil moisture for a pre-soaking of smut spores and the delay in their germination may mean less opportunity for infection of the wheat seedlings.

The work of Noble (13) shows that the spores of flag smut will only germinate after three days' soaking in water and then with difficulty. The addition of small quantities of the tissue of wheat seedlings to spores which had been presoaked in water for several days increased germination. The stimulation by the tissue is greater when the spores have been presoaked for three or more days than when water and fresh tissue are provided simultaneously. Griffiths (4) stated that the spores of flag smut do not germinate readily; but a fairly high percentage of germination occurred by floating the spores on distilled water in a watch glass at a temperature of 18° to 20° C. Germination began in two or three days and proceeded rapidly from the fourth day onwards. Fairly good germination also occurred in distilled juice expressed from wheat seedlings, while in some cases germinating seeds also proved a stimulating medium. Spore germination tests, which were made in our laboratory, confirmed the work of Noble in that the addition of wheat grain or wheat seedling juice to spores which had been presoaked in water for some days greatly increased germination.

This obviously will explain the difference in the incidence of the disease between the moist and the dry soil. The spores in the dry soil will receive water of irrigation just after sowing, *i.e.*, the spores and the seed will be in contact from the beginning. Germination of spores would be poor and chances of infection will, therefore, be slight. The spores in the moist soil, on the other hand, will be under different conditions; for they will have been presoaked for the requisite three days or longer, and will therefore, be stimulated into rapid germination as soon as the wheat grain is planted.

The germination of spores adhering to the grain coat will, also, be poor, as both spores and seed will be present together from the beginning.

Manurial Treatment

The influence of manuring on the severity of flag smut was studied by Forster and Vasey (3), who concluded that applications of lime up to 10 cwt. per acre markedly increased infection, as did applications of farmyard manure; when a mixture of both was applied, infection was particularly severe. Superphosphate appeared to have no effect on the disease. Millikan (9) made sand culture trials and concluded that calcium deficiency inhibited completely the development of flag smut in the susceptible variety Free Gallipoli, nitrogen and potassium deficiencies both tended to decrease the severity of the disease, and phosphorus and magnesium deficiences to increase it. He (10) also stated that a calcium excess of twice the normal dosage significantly increased infection in the Free Gallipoli variety; a four times normal excess had no effect; but severity of attack was increased by combining double calcium with one-fourth magnesium and one-tenth phosphorus. Excess of phosphorus or potassium alone had no effect. With the Ghurka variety no treatment increased susceptibility to any degree comparable with that of susceptible controls grown under the same conditions.

TABLE 2.—*The percentages of flag smut on Hindi D wheat grown at Dokki in 1941–42 and given different manurial treatments*

Treatment		Average smut infection per plot[a]	
Fertilizer	Amount per feddan	Number of smutted plants	Percentage
Control		299	39.9
Calcium nitrate	200 kilos	396	52.8
Potassium sulphate	Do	324	43.2
Superphosphate	Do	333	44.4
Lime	Do	352	46.9
Farmyard manure (Baladi)	20 cu. m.	367	48.9

[a] Each figure is an average for the six plots of a treatment.

In order to determine the effect of manurial treatment on the incidence of the disease, an experiment was carried out in 1941–1942 at Dokki, using 2×5 meters, artificially infested plots which had been differently manured. Each treatment was repeated six times. Artificially infested grains were used in all cases. The data in table 2 show that infection was severe in all cases, and that all the manures tried increased the incidence of the disease.

Smut Spore Load on the Seed

Millikan and Simo (11) stated that the use of a heavy spore load (1 per cent) for inoculum favored infection by *Urocystis tritici*. Two experiments on this phase of the disease cycle were conducted in 1940–1941 and 1941–1942 at Dokki using artificially infested grain. The smut spore loads added to the grain in 1940–1941 were: none, 0.001 gm., 0.01 gm., 0.1 gm., 1 gm., and 10 gm. In 1941–1942 they were: none, 0.1 gm., 0.5 gm., 1 gm., 5 gm., and 10 gm. per 1000 gm. of grain. Non-infested plots, 2×5 meters, were used and each treatment was repeated six times. Table 3 gives the results expressed as averages for six treatments. The data show that the incidence of the disease increases markedly with the increase of smut spore load on the grain.

TABLE 3.—*The flag smut infection in Hindi D wheat[a] infested with varying spore loads*

1940–1941			1941–1942		
Spore load per 1000 gm. of grain	Smutted plants per plot		Spore load per 1000 gm. of grain	Smutted plants per plot	
	Av. No.	Pct.		Av. No.	Pct.
None	2	0.3	None	68	9.1
0.001 gm.	9	1.3	0.1 gm.	252	33.6
0.01 gm.	25	3.3	0.5 gm.	349	46.6
0.1 gm.	57	7.6	1.0 gm.	404	52.5
1.0 gm.	75	10.0	5.0 gm.	580	77.2
10.0 gm.	149	19.9	10.0 gm.	615	82.0

[a] The grain was treated with a surface disinfectant before the flag smut spores were added.

Number of Waterings

Infection takes place when germinating flag smut spores come in contact with recently germinated wheat seedlings. It is, therefore, doubtful whether the amount of water given to the soil during the growing period of the crop influences the incidence of the disease. An experiment in 1941–1942 at Dokki tested the effect of this factor. Artificially infested, 2×5 meter plots were used. They were similar except for different numbers of waterings. Each treatment was repeated five times, and the results for each were averaged.

With a single watering at sowing time there were 487 smutted plants in a plot or 64.9 per cent infection; and with two waterings the infection was very similar, 492 smutted plants or 65.6 per cent infected. A slight increase, 71.9 per cent or 539 smutted plants, occurred when three waterings were given the plots. With four waterings, 67.2 per cent were smutted; and with five waterings, 69.3 per cent. The incidence of the disease was very high in all plots, and the number of waterings had no pronounced influence on the amount of infection.

VARIETAL RESISTANCE

It has been noticed during the study of this disease that certain varieties of wheat were susceptible to flag smut, while other varieties were comparatively resistant. An experiment at Dokki in 1941–1942 tested a number of commercial varieties with reference to this point. Artificially infested, 2×5 meter plots were used, and each treatment was repeated six times. Artificially infested seed and the "Herati" sowing method were used.

TABLE 4.—*The flag smut infection in eight commercial wheat varieties grown at Dokki in 1941–1942*

Variety of wheat	Smutted plants per plot	Infection
	Number	*Per cent*
Baladi 116	0	0
Baladi "Bouhi"	0	0
Kazouria "Taliani"	0	0
Hindi "Abiad"	201	26.8
Hindi 62 "Dahabi"	242	32.3
Mabrouk "Giza 121"	272	33.6
Hindi D.	325	43.3
Hindi Maarad.	343	45.8

The data in table 4 show that the Hindi and the Mabrouk varieties are very susceptible to flag smut, while the Baladi and the Kazouria varieties are immune.

USE OF FUNGICIDES FOR DISEASE CONTROL

Norwood (12) found that dusting the seed-grain with copper carbonate, Tillatin R, or V.T. 685 was ineffective against flag smut in the case of

heavily inoculated seed-grain. Good control was obtained by steeping the seed-grain in 1.5 per cent copper sulphate solution for three minutes or in a 1:400 formalin solution for ten minutes. Miller and Millikan (8) obtained from 80 to over 90 per cent control with copper carbonate on seed more heavily inoculated than seed normally used by farmers. Dawson (1) dusted wheat seed, heavily inoculated with *Urocystis tritici*, with copper

TABLE 5.—*Flag smut infection in Hindi D wheat grown from seed treated with fungicides and in plots on infested and noninfested soil at Dokki in 1940–41 and 1941–42*

Fungicide	Non-infested soil				Infested soil			
	1940–41		1941–42		1940–41		1941–42	
	Smutted plants per plot		Smutted plants per plot		Smutted plants per plot		Smutted plants per plot	
	No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
Control (not treated)	95	12.7	439	58.5	129	17.2	645	86.0
Flowers of sulphur								
5 gr./kilo seed	40	5.3	.....	.....	67	8.9	.....	.....
10 gm.	53	7.1	205	27.3	39	5.2	205	27.3
Copper carbonate								
5 gm./kilo seed	45	6.0	.....	.....	33	4.4	.....	.....
10 gm.	40	5.3	240	32.0	38	5.1	355	44.0
Agrosan G[a]								
2.5 gm./kilo seed	28	3.7	.....	.....	44	5.9	.....	.....
5.0 gm.	21	2.8	218	29.1	29	3.9	297	39.6
10.0 gm.	32	4.3	.....	.....	19	2.5	.....	.....
Uspulum[a]								
0.5 per cent	36	4.8	.....	.....	44	5.9	.....	.....
1.0 per cent	59	7.9	.....	.....	56	7.5	.....	.....
2.0 per cent	32	4.3	.....	.....	33	4.4	.....	.....
Germisan[a]								
0.5 per cent	69	7.9	176	23.5	41	5.5	323	43.1
1.0 per cent	39	5.2	.....	.....	50	6.7	.....	.....
2.0 per cent	56	7.5	.....	.....	40	5.3	.....	.....
Copper sulphate								
0.5 per cent	28	3.7	170	22.7	26	3.5	177	23.6
1.0 per cent	31	4.1	.....	.....	33	4.4	.....	.....
2.0 per cent	22	2.9	.....	.....	26	3.5	.....	.....
Lime-Sulphur								
0.5 per cent	201	26.8	...	.....	117	15.7	.....	.....
1.0 per cent	114	15.2	274	36.5	194	22.9	316	42.1
2.0 per cent	55	7.3	.....	.....	48	6.4	.....	.....
Bordeaux mixture								
0.25 per cent	85	11.3	.....	.....	101	13.5	.....	.....
0.5 per cent	45	6.0	.....	.....	87	11.6	.....	.....
1.0 per cent	48	6.4	287	38.3	45	6.0	335	44.7
Formalin								
0.5 per cent	21	2.8	124	16.5	19	2.5	132	17.6

[a] Mercury is the toxic ingredient of the fungicide.

carbonate (2 oz. per bushel) or dipped the seed in 1.5 per cent solution of copper sulphate and then in lime-water and got 4.33 per cent infection in the dry treatment, 7.3 per cent in the wet treatment, and 21.6 per cent infection in the untreated controls. The copper sulphate treatment impaired the vitality of the seed, and it is not recommended.

Two experiments were designed in 1940–1941 and 1941–1942 at Dokki

to determine the effect of certain fungicides in controlling the disease in Egypt. Artificially infested and noninfested plots, 2×5 meters, and artificially infested seed were used. Each treatment was repeated four times.

The following conclusions may be deduced from the data in table 5.

The two lower concentrations of lime-sulphur definitely increase susceptibility to disease.

The lower concentration of Bordeaux mixture is not satisfactory.

Flowers of sulphur, formalin, copper carbonate, Agrosan G, Upsulun, Germisan, copper sulphate, and the higher concentrations of lime-sulphur and Bordeaux mixture gave partial control.

There was more disease in plants on infested soil than in plants on noninfested soil.

We also determined the effect of the different fungicides on germination of the treated seed and growth of the seedlings. Formalin and the two higher concentrations of Germisan and copper sulphate had a bad effect on seed germination and growth. The other fungicides had no harmful effect on the plants.

SUMMARY

Flag smut is distributed throughout Egypt. It reaches its maximum in Lower Egypt (the Delta) and, then, decreases gradually southwards until it becomes rare in Upper Egypt. The intensity of the disease varies from year to year.

The loss caused by the disease in Egypt is small; it does not, usually, exceed 5 per cent, but under certain conditions it may become so destructive as to cause a loss of more than 50 per cent.

The plants of the early sowings are almost free from the disease; while those of the late sowings are badly attacked. Temperature appears to be the main factor responsible.

"Herati" sowing favors considerably the attack. Both depth of sowing and soil moisture at sowing play an important part in this connection. The seed is, usually, sown deeper in the "Herati" method than in the "Afir" method and the spores in the "Herati" are usually subjected to presoaking for a period of 8–15 days before sowing, while those in the "Afir" remain dry until sowing time, when the first irrigation is used. The disease increases with the increase in depth of sowing and with the presoaking of the spores before sowing.

No promising control was evident in manurial treatment of the soil. All manures tried increased the incidence of the disease.

The amount of water given to the soil during the growing period of the crop has no influence on the incidence of the disease.

The Hindi and the Mabrouk varieties of wheat are very susceptible to flag smut; while the Baladi and the Kazouria varieties are immune.

Partial control of the disease with no harmful effect on germination of the treated seed and growth of the plants is given by the use of 0.5 per

cent Germisan, 0.5 per cent Upsulun, flowers of sulphur, copper carbonate, and Agrosan G. "Afir" sowing, clean seed and soil, and early sowing are highly recommended. Cost of treatment with any of the recommended fungicides is very small and negligible.

The incidence of the disease increases with the increase of smut spore load on the seed.

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STEM-END ROT OF STRAWBERRIES

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INTRODUCTION

The study of various fruit rots of strawberries has commanded the attention of a large number of investigators wherever strawberries are grown and particularly in the United States. A number of organisms have been connected with the spoilage of the fruit in the field, in transit, and on the market. The most important of these, it is generally agreed, are various species of *Botrytis* of the *cinerea* type causing gray rot, and *Rhizopus nigricans* causing leak. Both diseases are considered serious in the United States (6, 19, 26, 27, 28), and have also been reported from Canada (4, 9) and Australia (1, 22), while damage from *Botrytis* is important commercially also in Argentina (15), Germany (12), Japan (13), New Zealand (5), Norway (14), and Russia (24). A more thorough search of the literature would without doubt reveal many more reported instances of these organisms attacking strawberries in other parts of the world.

In addition to these two well known rots, tan rot (*Pezizella lythri*), hard rot (*Rhizoctonia* sp.), and leather rot (*Phytophthora cactorum*) are common, especially in the United States (6, 10, 20, 21, 28), though not so prevalent as the first two previously mentioned. *Mycosphaerella fragariae*, the well known cause of leaf spot, was found to cause "black seed" disease of strawberries, particularly in Maryland and North Carolina according to Demaree and Wilcox (11) who first described this disease. A *Septoria*, however, presumably the conidial stage of *M. fragariae*, had been previously reported from England by Ogilvie in 1932 (17) as the cause of a hard rot of strawberries. Finally, *Sclerotinia sclerotiorum* is listed on strawberry fruits in the United States (21), *Didymella lycopersici* has been reported (23) as attacking flowers and fruits as well as leaves and roots of strawberries in England, and *Sclerotium rolfsii* to cause a soft rot in Florida (16). Stevens and Peterson (25) described a fruit rot of strawberry which they found first in Louisiana in 1914, and which, they state, was later found to be very common on berries in the market at Urbana, Illinois. They described the causal organism, a pycnidial fungus, as *Sphaeronemella fragariae*. These seem to be the only organisms which have been associated with fruit rots of strawberry.

FIELD AND LABORATORY OBSERVATIONS

In connection with some spraying experiments which had been started by the junior author, the strawberry plantation of Mr. Max Smith, near Lawrence, Michigan, was visited on July 2, 1947. A number of berries of the Robinson variety with disease symptoms were collected outside the experimental plots. Each was wrapped in newspaper separately, and placed with

others in a paper sack. Upon return to the laboratory the same evening, the sack was placed in the refrigerator.

On July 3, several of the berries were examined under the binocular dissecting microscope in an attempt to diagnose the various rots symptomatologically. Such diagnosis was verified when there was any doubt, by isolations from the diseased tissues. As the examination of the berries continued, it was noted that a comparatively large number had a brown discoloration accompanying a general dry rot which seemed to begin at the stem end. Further examination revealed the pycnidia of a fungus forming on the berries below the calyx. These pycnidia contained spores closely resembling those of *Dendrophoma obscurans*, the cause of leaf blight.

Up to July 7, fifty berries in all had been individually examined. Of these, 33 bore pycnidia. The prevalence of a fruit rot presumably due to *Dendrophoma obscurans* was interesting, as the fungus, well known as the cause of leaf blight in many localities in the United States as well as in Argentina (8), and new South Wales (3), has never been connected with a fruit rot as far as the writers have been able to determine. Pycnidia of *Dendrophoma* were also frequently observed on living or dead calyces and on the dead peduncles of the berries under scrutiny. It appeared as though the infection was started in the field on the peduncles and entered the berry after killing the stem.

The prevalence of *Dendrophoma* on the berries was emphasized when the examination of all the berries, 1215 in number, representing two pickings from six small experimental spray plots on Mr. Smith's plantation was completed. The first picking took place on July 4, 1947, and the second on July 7. These two pickings will be referred to as Lot I and Lot II, respectively.

The usual well known rots were represented in these two lots. In addition, a "stem-end rot" and a "soft rot" neither of which showed any external signs of a fungus, were found, as well as the "Dendrophoma rot" characterized by the presence of the pycnidia. Because it was suspected that all three of these may be stages of the same disease, all berries were returned to their boxes after examination, each group separated by a layer of newspaper, and kept in the laboratory for examination at a later date.

The initial examination of the fruit was completed on July 9 for Lot I, and July 10, for Lot II. On July 11 and 12 respectively, the two lots of berries were reexamined. As was suspected, it was found that a large number of the fruits previously classified in the groups "stem-end rot" and "soft rot," had developed pycnidia of *Dendrophoma*.

A final examination of the berries affected with "stem-end rot" and "soft rot" on July 11 and 12, was made on July 14 (Table 1).

Thus, 618 strawberry fruits out of 1215 picked and individually examined, developed pycnidia of *Dendrophoma*. This represents 50.8 per cent of all berries picked, and 76.3 per cent of all diseased berries in the two lots. It is not improbable that the 48 berries which were still classified in the

"stem-end rot" and "soft rot" categories at the end of the observations, would eventually develop pycnidia. The seriousness of the disease in these plots is evident from the above figures.

TABLE 1.—*Incidence of fruit rot in two lots of Robinson strawberries picked July 4 and 7, 1947, respectively, near Lawrence, Michigan*

Condition of berries	Lot I			Lot II		
	July 9	July 11	July 14	July 10	July 12	July 14
Sound	239	239	239	171	171	171
Pycnidia of *Dendrophoma*	87	272	289	159	292	329
"Stem-end rot"	161	32	16	63	11	3
"Soft rot"	69	15	14	125	44	15
Gray rot (*Botrytis* sp.)	36	36	36	30	30	30
Tan rot (*Pezizella*)	14	14	14	52	52	52
Leather rot (*Phytophthora*)	5	5	5	1	1	1
Leak (*Rhizopus*)	1	1	1	0	0	0
Blue mold (*Penicillium*)	1	1	1	1	1	1
Total	613	615[a]	615[a]	602	602	602

[a] Two berries having both *Dendrophoma* pycnidia and *Botrytis* conidiophores, are included twice.

THE DISEASE

Stem-end rot of strawberries appears to invade the fruit from infected peduncles. The most typical symptom is a brownish discoloration of the upper part of the berry, at first under the calyx, accompanied by a softening of the tissues. The softening of the tissues eventually spreads to the whole fruit giving rise to a general soft rot. The berry gradually begins to shrivel, the portion beneath the calyx drying and often developing into a depression. Pycnidia of *Dendrophoma obscurans* develop under the epidermis, become erumpent and exude large quantities of pycnidiospores from the ostiole. The pycnidia are at first hidden from view by the calyx lobes but later develop on other parts of the berry as well. The fruit eventually mummifies.

IDENTIFICATION OF THE ORGANISM

Upon tentative identification of the organism as *Dendrophoma obscurans*, strawberry leaves of the Dunlap variety with typical symptoms of *Dendrophoma* leaf blight were collected at East Lansing, Mich., brought to the laboratory, and placed with their petioles in water under a bell jar. A few days later they were examined for pycnidia. Many pycnidia were found which answered the description of *D. obscurans*. Conidiophores and spores from leaves, berries, calyces, and peduncles, corresponded closely; pycnidia on leaves, however, tended to be small and dark. Pure cultures obtained by transferring pycnidiospores from berries, leaves, and calyx lobes, to maltose agar, and from plantings of diseased berry tissue taken from the inside of berries as far away as possible from the location of the pycnidia, were in all ways similar. All produced pycnidia and pycnidiospores identical with those on various parts of the host.

THE PERITHECIAL STAGE

Of various cultures on maltose agar inoculated on July 3, from the berries picked on the previous day, one labelled S6 proved particularly interesting. Diseased tissue from a berry on which no external signs of a fungus were as yet evident, but which exhibited symptoms of stem-end rot, had been planted on five places in a Petri dish containing maltose agar. Of the five colonies developed, one gave rise to typical sporodochia of *Pezizella lythri,* while the other four eventually developed round, black fruiting bodies with long beaks. On July 17, one of these structures gave forth, when crushed, a large number of immature asci. Pycnidia interspersed among the perithecia contained

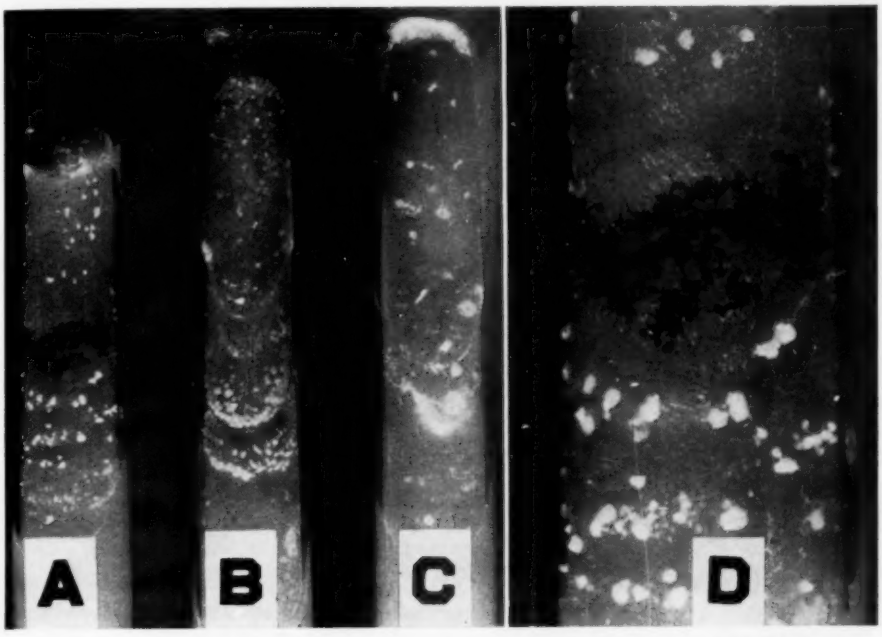

FIG. 1. Three single pycnidiospore cultures (A, B, and C) of *Dendrophoma obscurans,* on corn meal agar, isolated from (A) strawberry leaf with typical symptoms of leaf blight, (B) infected strawberry calyx, and (C) infected strawberry fruit. Note band of perithecia among the pycnidia in (A) and in (D) which is an enlarged view of (A). Subsequent transfers on corn meal agar from (B) and (C) also produced an abundance of perithecia. Photographs by F. C. Strong.

pycnidiospores typical of *Dendrophoma obscurans.* The connection between the two stages appeared probable. Six other isolations from different berries yielded pure cultures of *D. obscurans,* with an abundance of pycnidia, but no other stage except a single black fruiting body with two long beaks, similar to the perithecia in culture S6, buried in the agar of culture S15.

On the same day, beaked structures similar to those found on agar, were discovered on the calyx of a berry which had been kept in a moist chamber for approximately two weeks. Under the binocular dissecting microscope, black, bristle-like structures, the beaks of perithecia buried in the calyx

tissue, were seen protruding through the epidermis. The globose base was easily dissected out of the host tissue and when crushed in water under a cover glass gave forth an oil exudate together with some cellular matter indicating the beginning of ascus formation. On the following day, more perithecia were found on the calyces and peduncles of berries which had been kept in the refrigerator, wrapped in paper since July 2. Definite asci with immature spores issued from one that was crushed. Later in the season (Sept. 9), fully mature ascospores were found in perithecia that had developed on the strawberries in a moist chamber. All berries, on the calyces of which perithecia were found, were heavily infected with *Dendrophoma obscurans*, as evidenced by the abundant pycnidia at the stem end. Pycnidia were also invariably associated with the perithecia on the calyces and the peduncles.

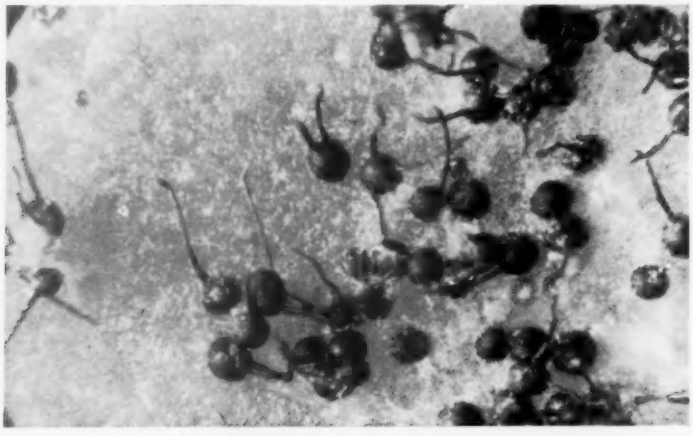

FIG. 2. Perithecia of *Gnomonia* sp., the perfect stage of *Dendrophoma obscurans*, on corn meal agar. ×69. Photograph by F. C. Strong.

Anderson (2) who searched leaves diligently for the perfect stage of *Dendrophoma obscurans* from September to May, failed to find it. He suggests that since the conidia are able to overwinter, a perfect stage would not be necessary for the survival of the fungus. The writers have not searched for perithecia on leaves. The only perithecia found in the field were collected on July 21 on the calyces and peduncles of two small, mummified berries in a Dunlap strawberry patch in East Lansing.

Several single pycnidiospores were now isolated from leaves, calyx lobes, and berries, in an attempt to obtain proof of the connection between *Dendrophoma obscurans* and the new ascomycete. The colonies resulting from all of these were in all respects similar. On July 24, transfers were made to corn meal agar slants and stored away. When they were examined on September 10, both perithecia and pycnidia had been formed abundantly by two cultures, DL2 2 and DL4.2, both derived from single pycnidiospores isolated from leaf tissue. The pycnidia were scattered over the slant, but

the perithecia were crowded together forming a black band at the base of the slant (Fig. 1, 2).[1] All other slants had produced pycnidia but no perithecia. Subsequent transfers from cultures originally isolated as single pycnidiospores from calyx, berry, and leaf, have produced an abundance of mature perithecia on corn meal agar in Petri dishes (Fig. 2). No perithecia have ever been found on the few cultures on potato-dextrose, oat, plain (nutrient), or maltose agar, with the exception of the two plates of S6 and S15 on maltose, previously mentioned. This experience explains why Anderson (2), who worked with oat agar, failed to produce the perfect stage in culture. Experiments are in progress to determine the factors affecting perithecial production.

In old Petri-dish cultures containing perithecia, the ascospores are released from the perithecium through the long, beaked ostiole, in a droplet of liquid which either remains as a glistening droplet at the tip, or rolls part way down, often forming a collar around the neck. Pure cultures were easily obtained by touching a sterile platinum needle to the droplet containing ascospores, and transferring them on agar. Such cultures gave an abundance of pycnidia of *Dendrophoma obscurans* on potato-dextrose, and oat agar, and the colonies were indistinguishable from those derived from pycnidiospores planted at the same time on the same types of media.

The cultural experiments, described above, are offered as evidence of the connection between *Dendrophoma obscurans*, and the perithecial stage herein discussed.

CLASSIFICATION OF THE PERFECT STAGE

The characters of the perithecial stage, place the organism in the genus *Gnomonia* of the Gnomoniaceae in the Sphaeriales. The black, globose perithecia, are deeply buried in the host tissues from which their long necks project; the walls are more or less leathery; there is no stroma. Asci are formed, but when the spores mature, the ascus wall deliquesces and the ascospores are released in a droplet of liquid through the ostiole. When perithecia containing mature or very nearly mature ascospores are crushed, the spores released cling together in the same position in which they were formed in the ascus, but no ascus wall can be demonstrated. Application of pressure on the cover glass will cause the ascospores to separate. They do so with difficulty, however, because they are held together in a viscid matrix.

The ascospores are one-celled at first, but a septum develops by the time they are released from the perithecium, dividing them into two unequal cells. The spores are curved. They are characterized by five to seven rather large refractive droplets, the presence of which makes it difficult to distinguish the septum. Most spores examined had three of these droplets in each cell. Old spores become highly vacuolated.

Perithecia from corn meal agar measure 236–545 μ in diameter, the

[1] The writers are indebted to Professor F. C. Strong of the Department of Botany and Plant Pathology, Michigan State College, for the photographic work in connection with this investigation.

length of the neck varying from 600 μ to 1455 μ. Ascospores from the same source, measured 7–11.5 $\times$ 2–3 μ. The species of *Gnomonia* to which the fungus belongs has not been determined as yet. Studies of the fungus in culture, as well as host inoculation experiments are in progress and will be reported as soon as sufficient data are on hand.

DISCUSSION

The perithecial stage of *Dendrophoma obscurans*, as reported herein, appears to be very similar to that of *Zythia fragariae*, the cause of strawberry leaf blotch found in England by Wormald (29). Strawberry leaf blotch was described in 1941 by Wormald and Montgomery (31, 32) and was at that time ascribed to *Phyllosticta grandimaculans* Bubak and Krieger (7). In spite of the great resemblance, neither Bubak nor Petch (18) who identified Wormald's fungus as *Zythia fragariae* Laibach (*Phyllosticta grandimaculans* B & K), mentioned any resemblance to *Dendrophoma obscurans* (E & E) Anderson. Nor does Wormald mention the American fungus which he obviously considers distinct. With similar perfect stages discovered for the two organisms, a comparison of material from England and America should prove interesting. It is of further interest to note that *Sphaeronemella fragariae* St. et Pet., found on strawberries by Stevens and Peterson (25) is also a pycnidial fungus and that it has many characters in common with both *Zythia fragariae* and *Dendrophoma obscurans*. According to the description, it differs from these two chiefly in the size of the pycnidia—an admittedly variable character—and in the fact that its conidiophores are described as simple. Simple as well as branched conidiophores, however, are described for *Zythia fragariae* by Wormald, and are common with our fungus. Although Stevens and Peterson grew their organism on corn meal agar, no perithecial stage was reported.

In his discussion of Dendrophoma leaf blight of strawberry, Anderson (2) states: "While the disease causes serious loss of functioning leaf tissues, it is not considered of sufficient economic importance to warrant spraying or other expensive control measures." The same opinion is expressed by Demaree (10). If further observations show that stem-end rot of strawberries is as severe elsewhere as it was determined to be in the small experimental plots near Lawrence, Mich., and as it was observed on Dunlap in East Lansing in 1947, it may be very much worthwhile economically to devise control measures for this disease.

SUMMARY

A disease described as stem-end rot of strawberries, presumably caused by *Dendrophoma obscurans*, the cause of strawberry leaf blight, was found to cause severe damage to strawberry fruits in some plots under observation near Lawrence, Michigan, in 1947. As much as 50.8 per cent of all fruits picked from six small experimental plots were affected. The organism isolated from fruits, calyces, and peduncles of diseased strawberries yielded

colonies and fruiting bodies on agar indistinguishable from those produced by isolations of *Dendrophoma* from typical leaf blight lesions.

A perithecial stage assigned to *Gnomonia* was discovered on agar and on calyces and peduncles of strawberries in association with the pycnidia of *Dendrophoma obscurans*. Pycnidiospore plantings on agar yielded perithecia, and ascospore sowings yielded pycnidia. The species to which the perfect stage belongs has not been determined as yet.

The similarity between *Dendrophoma obscurans* and its perfect stage on the one hand, and *Zythia fragariae* and its *Gnomonia* perfect stage in England on the other, are briefly discussed.

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TWO INTERESTING LEAF SPOTS OF FIG

E. C. Tims and L. S. Olive

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INTRODUCTION

In September, 1939, a very conspicuous leaf spot was observed on a fig tree (*Ficus carica*), Celeste variety, in southern Louisiana. During the summer and fall of the following year, it was observed in two other localities. In 1942, the same disease was found in several widely scattered locations in the State. The first infections were found late in the season and were apparently of little importance, but in 1942, a small group of heavily infected fig trees bearing immature fruit was found near Baton Rouge in early July. These trees were partially defoliated and quite unsightly in appearance. Since this leaf spot was apparently different from any previously described on fig, it was investigated further. The disease was designated as "Cephalosporium leaf spot" (6).

About the same time that the Cephalosporium spot was found, another very similar leaf spot was observed on fig trees in the same localities. The spots were so similar in general appearance that at first they were considered to be due to the same fungus. Later observations indicated, however, that the second spot was somewhat different from the one caused by *Cephalosporium*. The fungi associated with the two leaf spots were entirely different.

The two leaf spots developed at about the same time, usually in July or early August, often on the same tree and occasionally on the same leaves. The Cephalosporium spots could be distinguished by the presence of cottony white tufts of a fungus on the lower surface, while the lower surface of the spots of the second disease had a smoky, brownish gray fungus growth in the center surrounded by a ring of white mycelium.

Apparently neither of these leaf spots on fig had been previously described. A "zoned" leaf spot of coffee associated with *Cephalosporium* sp. was reported by Fawcett (2) from Puerto Rico in 1915. In its early stages the spot illustrated in his paper is similar in appearance to the one on fig. Fawcett isolated the causal fungus and reproduced the disease with pure cultures of the *Cephalosporium*. Later Abbott (1) reported a zonal leaf spot of coffee in Peru which he attributed to *Cephalosporium*. Neither of these workers gave a specific name to the fungus.

The two new leaf-spots of fig are described in this paper and their causal organisms are named.

THE CEPHALOSPORIUM SPOT

(Fig. 1, A and B; Fig. 2, A–D)

Symptoms

The small, circular, brown spots first appear on the upper surface of the

leaves. As they enlarge a series of irregular concentric rings is formed, light brown bands of dead tissue alternating with darker brown rings. The spots may enlarge to form as many as a dozen rings. The centers of the spots often fall out, giving the leaves a ragged appearance. Soon after the spots appear, the characteristic small fluffy, cottony, white tufts of mycelium develop on the lower surface in large numbers. These cottony tufts are made up of cords of hyphae that have surrounded the leaf hairs. The small cephalosporia (heads) with numerous small, hyaline, single-celled conidia are produced on slender phialides projecting from the cords of hyphae (Fig. 2, B–D). The white tufts persist on the lower surface of the spots until the dead areas collapse and fall out. A typical infected leaf is shown in figure 1, A and B.

Technical description

Cephalosporium fici sp. nov.

Maculis manifestis, 1–6 cm. diametro, in superficie superiori zonatis, auctione subalba plumosaque in superficie inferiori tectis. Hyphis per partes superiores pilorum folii se glomerantibus ut funes hypharum fiant qui phialides in ipsis vel in ramis brevibus lateralibusque efficiant. Phialidibus gracilibus, septum in basibus frequenter atque septum unum vel duo supra habentibus, 2.4–4.0 × 34–99 μ. Conidiis singulis in apicibus phialidum effectis, se in globos 6.9–12.2 μ diametro glomerantibus; conidiis ovatis usque ad forman cylindricam, rectis vel subcurvatis solam cellulam hyalinam, 1.9–4.5 × 3.8–10.4 μ mediis inter maximum minimumque 3 × 6 μ, habentibus.

Parasiticum in foliis, *Fici caricae.*

Spots very conspicuous, 1–6 cm. in diameter, distinctly zonate on the upper surface of the leaf, with light brown bands of dead tissue alternating with more narrow and darker brown rings, lower surface covered with a whitish fuzzy growth of the fungus and densely stippled with white dots where the sporulating cords of hyphae have climbed the leaf hairs; dead parts of the leaf spots frequently falling out.

Internal mycelium intercellular, hyaline, giving rise to the external mycelium through the stomates, the external mycelium hyaline, hyphae climbing the leaf hairs and massing together along the upper portions of the trichomes to produce compact cords of hyphae, measuring 18–55 × 250–400 μ, giving rise to phialides directly or from short lateral branches. Phialides slender, tapering gradually towards the apex, each phialide frequently cut off by a cross wall at its base, 1–2-septate or non-septate above, measuring 2.4–4.0 × 34–99 μ. Conidia produced singly at the tips of the phialides but aggregating into globose masses measuring 6.9–12.2 μ in diameter, conidia oval to cylindrical, straight to slightly curved, 1-celled, hyaline, measuring 1.9–4.5 × 3.8–10.4 μ, averaging 3 × 6 μ. Parastic on leaves of *Ficus carica;* E. C. Tims, Schriever, Louisiana; September 17, 1947.

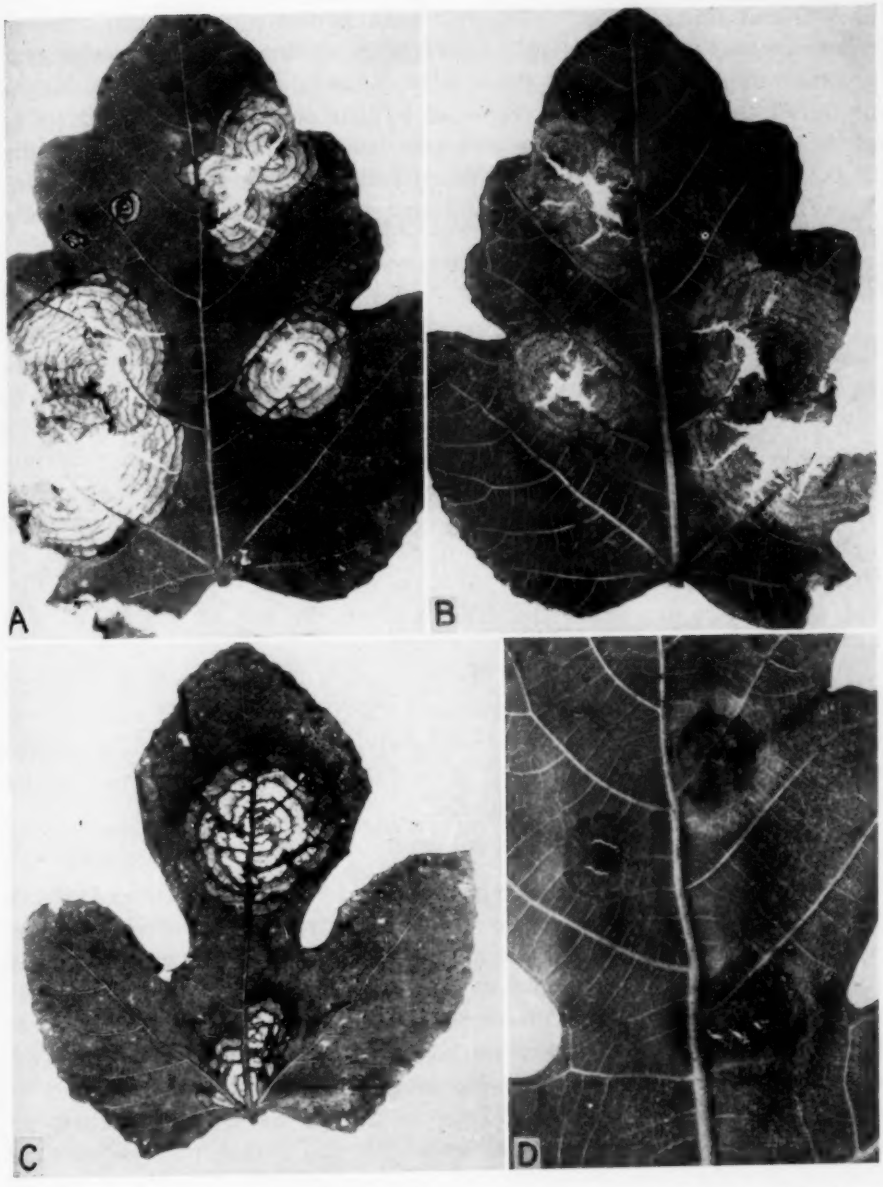

FIG. 1. The two leaf spots of fig. A and B, *Cephalosporium fici;* C and D, *Ormathodium fici.* A, upper surface; B, lower surface of the same leaf, natural infection. C, upper surface of the Ormathodium spot, natural infection; D, a view of the lower surface of an artificially infected leaf.

Cultural characters

Cephalosporum fici was isolated from typical lesions on fig leaves, both by tissue transfers and spore dilutions. The fungus grew well and produced numerous spores on several culture media. On Czapek's agar

growth was white, becoming slightly pink as the cultures aged. Dense, raised, cottony masses of mycelium developed on this agar. On water agar growth was appressed, with practically no aerial mycelium. The fungus produced characteristic large ropes of hyphae on string bean agar. One of the smaller cords of hyphae with whorls of phialides is shown in figure 2, D. Conidia were borne in heads on slender tapered phialides (Fig. 2, A). They were similar in size and shape to the conidia taken directly from diseased fig leaves.

Taxonomy

When the fig leaf spot was first observed (6), the causal fungus was thought to be *Cephalosporium acremonium* (Corda). Later examination, however, showed that the fig fungus differed in some respects, notably in spore size, from the type *C. acremonium* associated with the black-bundle disease of corn. Reddy and Holbert (4) made a study of *C. acremonium* in which they discussed the literature rather thoroughly. The spore measurements and illustrations of spores of *C. acremonium* as given by these writers are distinctly different from those of the fig fungus. The fig *Cephalosporium* resembles *C. carpogenum* Ruehle as described by him (5), but again there are important differences in the size of conidia as well as the size of phialides.

The *Cephalosporium* causing the leaf-spot of fig is apparently different from any previously described species. For this reason and also in view of the fact that no species of *Cephalosporium* or morphologically similar fungi have been previously reported on *Ficus carica,* the writers consider it best to regard this as a new species.

Pathogenicity

Mature fig leaves on orchard trees were successfully inoculated in two ways during the summer months: (1) by spraying them with spore suspensions, and (2) by placing small pieces of a bean pod culture of the fungus on the lower leaf surface. After the latter type of inoculation, the leaves were wrapped in moist paper and covered with paper bags to hold in the moisture for 24 to 48 hours. During periods of warm, wet weather in July or August, symptoms appeared on leaves inoculated by each of the above-mentioned methods within 4–6 days, and the spots enlarged rapidly, reaching a diameter of 3–5 cm. within two weeks. The centers of such spots often fell out, leaving ragged, irregularly shaped holes in the leaves similar to those observed in naturally infected leaves.

Apparently most infection takes place through the lower surface of the leaves, although occasionally infection was produced by inoculating the upper surface. Within a week after infection, the small white fruiting structures of the fungus were observed on the lower surface of the leaf. Pure cultures of the *Cephalosporium* were reisolated by making spore dilutions from such material.

Primary inoculum probably comes from old infected leaves on the

ground, since the first infected leaves are invariably those nearest the ground. Early in the summer of 1940 some inoculations were made with *Cephalosporium fici* on a small isolated group of fig trees several miles away from any known Cephalosporium-infected trees. Later in the season the fungus spread to other trees nearby. Natural infection occurred on all the trees in the group the following summer.

THE ORMATHODIUM SPOT

(Fig. 1, C and D; Fig. 2, E–J)

Symptoms

The spots vary from 1 to 8 cm. in diameter and are roughly circular. They first appear as small round, dark brown lesions that gradually enlarge, forming definite concentric rings on the upper surface of the leaf (Fig. 1, C). The older portions of the spots fade out and soon become papery white. There may be from 2 to as many as 12 concentric rings formed in a single spot, the older rings in the center becoming almost perfectly white with age. The outer ring may vary from light brown to a very dark brown. The dark outer rings usually appear when the spots are young and growing rapidly. In the old spots the white papery centers often fall out and leave ragged holes in the leaves.

The lower surface of the spots also has a characteristic appearance (Fig. 1, D). The centers are usually brown in the younger spots, with distinct whitish borders where the fungus mycelium is developing on the surface. The brown center with the white mycelium around the border is characteristic of this leaf spot. As the spots become older the smoky-grayish brown masses of conidia and conidiophores of the causal fungus usually appear in the centers. Microscopic observation reveals the presence of numerous septate and much-branched conidiophores bearing curved, septate conidia (Fig. 2, E–G). This development of conidia does not always occur, but when moisture is plentiful they are usually found. Some fig trees have been observed throughout an entire season with numerous spots on the lower leaves, but few conidia were produced on any of them.

The spots seldom appear on the fig trees in south Louisiana before early July and are usually not conspicuous until later in the summer. They generally show up on the very lowest leaves and seldom appear on the upper half of the tree. The disease was very conspicuous on some of the affected fig trees in late August. Practically all the lower leaves had from one to several of the large spots on them.

Technical description

Ormathodium fici sp. nov.

Maculis magnis, brunneis, aliquanticulum concentrice zonatis. Mycelio intra folium inter cellulas sito, hyalino; mycelio externo subbrunneo, 2–4.5 μ diametro. Conidiophoris subbrunneis, ramosis, multiseptatis, nodos manifestos habentibus, in quibus conidia feruntur. Illa 3–4 $\times$ 135–240 μ

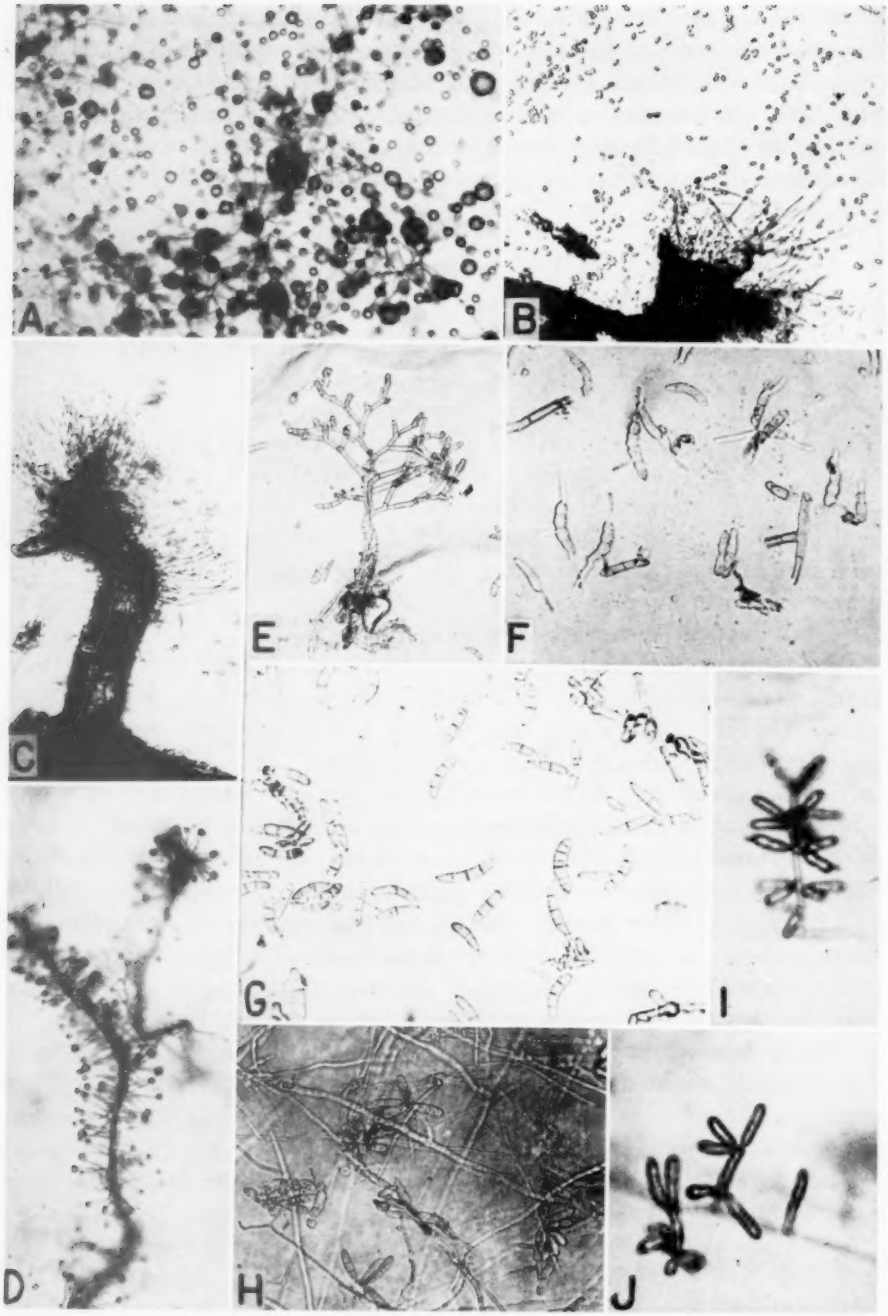

FIG. 2. *A–D, Cephalosporium fici.* A, Cephalosporium heads in water droplets on string bean agar, 14×; B, conidia directly from fig leaf, 146×; C, leaf hair with fungus growing over it, 146×; D, ropes of hyphae with phialides and spore heads developed on potato-dextrose agar, 62×. *E–J, Ormathodium fici* (E to G, directly from fig leaf). E, conidiophore with typical branching, 140×; F, germinating conidia, 280×; G, conidia, 280×; H, mycelium and conidia from water agar culture, 280×; I–J, conidia attached to conidiophores, developed on aerial mycelium in water agar culture, 280×.

semi-erecta et pila foliorum circumplectentia praecipue se sustenent. Conidiis paucis vel multis in racemis ad apices conidophorum ac a latere in nodis per typum effectis, singulis exsistentibus aut in seriebus brevibus duo vel tria numero continentibus, cylindricis vel clavatis, rectis usque ad formam perspicue curvatam, subbrunneis, 1–5 (–7) septatis, levibus vel per occasionem minute verruculosis 3.4–5.2 × 13.9–29.6 μ, hila minuta in finibus proximis gerentibus, potestatem tubuli germinum e quaque cellula efficiendi habentibus.

Parasiticum in foliis *Fici caricae.*

Spots large, brown, appearing somewhat concentrically zoned on the upper leaf surface and covered with a grayish fuzzy growth of the fungus on the lower surface, dead parts of the lesions frequently falling out. Mycelium within the leaf intercellular, giving rise to the external mycelium through the stomates; external mycelium light brownish, 2–4.5 μ in diameter. Conidiophores arising chiefly from the external mycelium, brownish, branched, multiseptate, with conspicuous nodes on which the conidia are typically borne in groups, semi-erect and supporting themselves mainly by twining around the leaf hairs, measuring 3–4 × 135–240 μ. Conidia typically produced in clusters of few to many at the tips of the conidiophores and laterally on the nodes, arising singly or occasionally in short series of 2–3, leaving minute wart-like scars at the points where they are shed from the nodes, cylindrical or clavate, straight to distinctly curved, light brownish, 1–5-(rarely up to 7–) septate, the majority 3-septate, smooth or occasionally becoming minutely warted, measuring 3.4–5.2 × 13.9–29.6 μ, possessing a minute hilum at the proximal end, producing a germ tube from any cell.

Parasitic on leaves of *Ficus carica;* E. C. Tims, Schriever, Louisiana; September 17, 1947.

Cultural characters

Ormathodium fici was isolated by making spore dilutions with string bean agar. The conidia germinated rather slowly at room temperature. The germinating spores were picked up from the culture plates and transferred to agar slants. Numerous attempts were made to isolate the fungus from small pieces of fig leaf with mycelium attached, but without success.

The fungus grew slowly on agar media. Colonies grown at 28°–32° C. for 38 days on oat meal agar were about 5 cm. in diameter. Growth was somewhat faster at 22°–25° C. The aerial mycelium was almost pure white. But the colonies on oat meal agar were light to dark brown. Sporulation was very sparse on most culture media. Occasionally conidia were observed in short chains of 2 or 3. Many cultures failed to produce conidia during several months of growth on a number of different media. Some sporulation occurred on water agar plates (Fig. 2, H–J). Certain strains of the fungus that produced no conidia in culture proved to be parasitic on fig leaves, where normal sporulation took place.

Taxonomy

The genus *Ormathodium* was erected by Sydow (1928) to accommodate a parasitic *Dendryphium*-like fungus. As was recently pointed out by one of the writers (3), *Dendryphium* was founded on a saprophytic species and practically all species placed in the genus since then have been saprophytes. Both *Dendryphium* and *Ormathodium* are dematiaceous genera characterized by cylindrical, several-septate conidia borne in chains or sometimes singly on branched conidiophores. *Ormathodium* is distinct primarily in its decidedly parasitic nature.

The new species on fig leaves has a large percentage of its conidia borne singly on the conidiophores, but conidia are also occasionally found in short chains of 2–3 on the leaves and more frequently so in the cultures. The fungus might fit just as well into the genus *Dendryphiella,* which has conidia borne singly on nodes of the conidiophores, but this genus was erected to accommodate a saprophytic species. The writers are aware of the fact that genera cannot always be adequately separated on the basis of parasitism and saprophytism. Nevertheless, it is believed that these factors should be employed in the taxonomy of Fungi Imperfecti wherever they can be used to advantage. The new species is therefore included in the genus *Ormathodium,* at least until a better system of classification for this group of fungi has been devised.

The present fungus in many respects resembles *Ormathodium ambrosiae* Olive (3), recently described as a parasite on leaves of *Ambrosia trifida* in Louisiana. The latter species is characterized by the presence of very similar conidiophores supported by the leaf hairs and by having 1–5-septate conidia borne singly or in short chains of 2–3. Other characters, such as symptoms, cultural characteristics, and conidial size, show that *O. ambrosiae* is distinct from the species on fig leaves. A search through the literature has failed to reveal any parasite of the fig with characters of the present fungus.

Pathogenicity

Inoculations were made on fig leaves in the following manner. The fungus was grown on oat meal agar slants for about 3 weeks, after which the agar and fungus were macerated and made into a paste with the addition of sterile water. Small amounts of this material were placed on the lower surfaces of fig leaves, which were kept moist for 48 hours. Typical spots developed in 10 days to 2 weeks. The leaf shown in figure 1, D was inoculated by this method. The fungus reisolated from such spots was identical with the original isolate.

Another method of inoculation was as follows: Small sections of naturally infected leaf tissue with the fungus growing on them were attached to the lower surface of healthy fig leaves. The fungus soon grew from the diseased leaf tissue onto the healthy leaves, causing typical spots. All of the inoculations were made on fig trees growing out in the open during late summer months when conditions were favorable for infection.

SUMMARY

Two rather conspicuous leaf spots of fig are described and their causal fungi named. *Cephalosporium fici* nov. sp. causes a large concentric ring spot with white cottony tufts of mycelium and conidia on the lower surface of the leaf. *Ormathodium fici* nov. sp. produces a somewhat similar spot with grayish-brown fungus growth and a definite white border on the lower leaf surface. Both fungi when inoculated onto healthy fig leaves produced the diseases in typical form.

The writers are grateful to Dr. P. G. Moorhead, Head of the Department of Classical Languages, for his preparation of the Latin diagnoses.

Specimens of both of the above described species have been placed in the mycological collection of the United States Department of Agriculture, Beltsville, Maryland, and in the mycological herbarium at Louisiana State University, Baton Rouge, Louisiana.

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PHYSIOLOGIC SPECIALIZATION IN GUIGNARDIA BIDWELLII, CAUSE OF BLACK ROT OF VITIS AND PARTHENOCISSUS SPECIES[1]

E. S. LUTTRELL[2]

(Accepted for publication April 16, 1948)

A disease of Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.), known as black-rot leaf spot, has been recognized for many years. Stevens and Hall (6) mentioned it briefly in 1921, and in 1924 Burger (1) noted its occurrence in Florida. A similar disease was reported on Boston ivy (*Parthenocissus tricuspidata* (Sieb. and Zucc.) Planch.) in the District of Columbia by Edson (2) in 1937. The pathogen associated with each of these leaf spots has been considered to be *Guignardia bidwellii* (Ell.) Viala and Ravaz, the fungus that causes black rot of grapes.

During the past three years black rot has been observed on both Virginia creeper and Boston ivy at several locations in Georgia. Attention was centered on this disease by the complaint of a local nurseryman that muscadine grape stock had been refused certification because of the presence of black rot on Virginia creeper vines adjacent to his vineyard, this being considered a possible source of inoculum for the muscadine grapes. In view of the results of a previous study (3) which demonstrated that cross infection could not be obtained with *Guignardia bidwellii* on native bunch grapes and on muscadine grapes, it seemed unlikely that the fungus on Virginia creeper would serve as a source of inoculum for the disease on muscadine grapes. The investigations described here, therefore, were concerned chiefly with this problem. This report contains the results of cross inoculation experiments with the black-rot fungus on bunch grapes, muscadine grapes, Virginia creeper, and Boston ivy, and also includes a few notes on the black-rot disease on the latter two suscepts.

THE DISEASE

The symptoms of black rot on bunch grapes have been described previously by Reddick (4) and on muscadine grapes by Luttrell (3). It may be recalled that on bunch grapes black rot is primarily a disease of the fruit that results in the mummification of the infected berries. It also produces brown spots on the leaves and black cankers on the stems. On muscadine grapes it is primarily a disease of the vegetative parts characterized by severe leaf spotting and cankers of the stems and peduncles. It rarely occurs on the fruit of resistant varieties; and, even on susceptible varieties, it usually causes only superficial scabs and cankers. It should be noted that the question of whether this difference in symptoms may be

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I am indebted to Dr. B. B. Higgins, Georgia Experiment Station, and Dr. C. M. Tucker, University of Missouri, for their criticisms of the manuscript and to Mr. Miller Thompson, Conyers, Georgia, for furnishing material on several occasions.

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attributed to a difference in the suscept or to a difference in the pathogen has not been answered. Since the European vinifera grape is susceptible

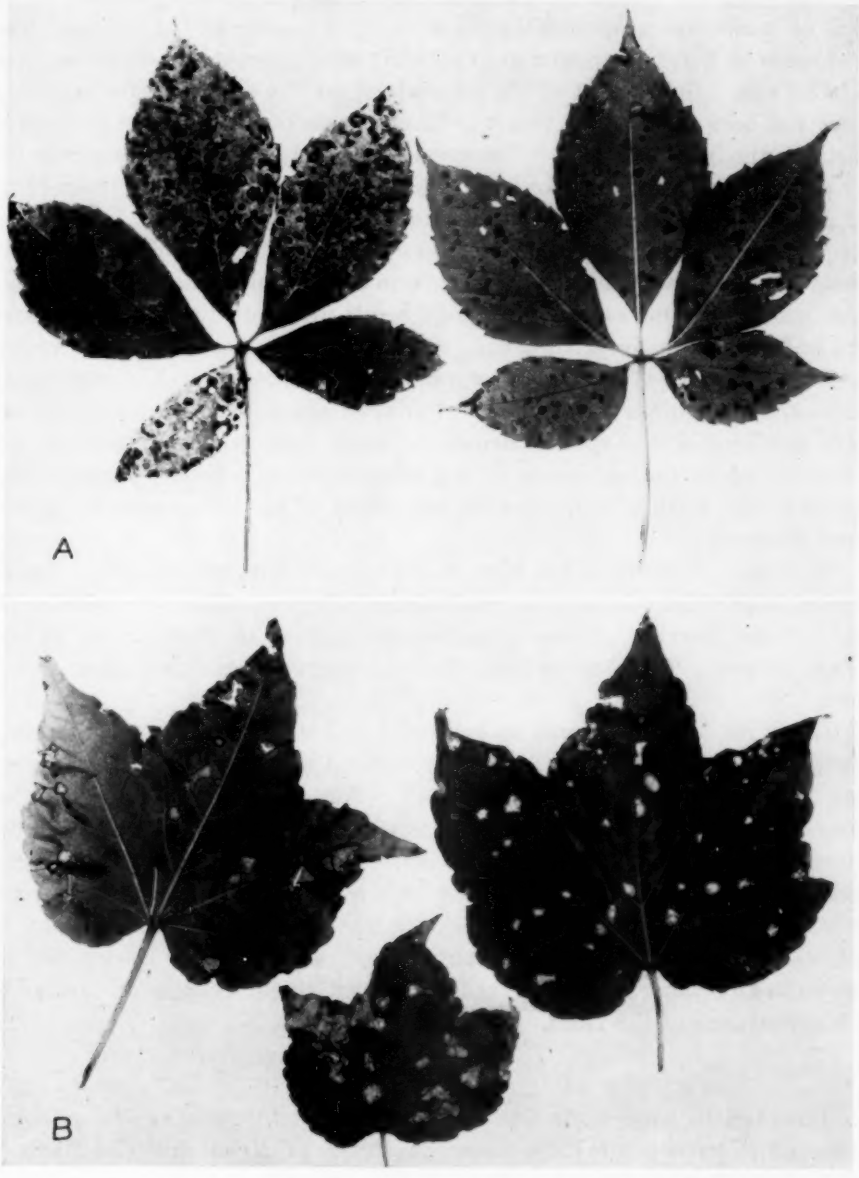

FIG. 1. A. Black-rot spots on leaves of Virginia creeper, at the left from the upper surface, at the right from the lower surface; ×1/2. B. Black-rot spots on leaves of Boston ivy, the leaf on the left from the lower surface, the two on the right from the upper surface; ×1/3.

to *Guignardia bidwellii* from both sources, it should be possible to determine this point by inoculating vinifera grape berries with the pathogen

from both native bunch grapes and muscadine grapes. Unfortunately, mature vinifera grape vines have not been available for this purpose.

The black-rot disease of Virginia creeper and Boston ivy is similiar to that of muscadine grapes in that it is chiefly a disease of the foliage. The leaf spots on Virginia creeper are relatively small, varying in diameter from 3 to 5.5 mm. Generally they do not coalesce, and in natural infections they have not been observed to result in the necrosis of areas of the leaf blade distal to the infected tissue. The spots are dark brown and are surrounded by a broad purplish-black border which merges with the brown center (Fig. 1, A). Pycnidia are produced sparingly on both surfaces of the spots. Brown cankers may sometimes form on the petioles. On Boston ivy the spots are larger, varying in diameter from 5 to 9 mm. They often coalesce and, when the veins are involved, may result in the death of large areas of the leaf beyond the infected tissue (Fig. 1, B). The spots are tan to brown and are surrounded by a narrow purplish-black border. The centers of older spots are often buff-colored. Pycnidia are produced abundantly on both surfaces of the spots. Brown to black cankers are formed on the petioles and on the peduncles of the influorescences. Severe cankers may result in the death of entire leaves, but blight of the inflorescences has not been observed.

Infection of the fruit has been found once on Virginia creeper. Small, brown, superficial, leathery lesions bearing a few pycnidia were present on the infected berries. These symptoms are similar to those on muscadine grape berries. Stem lesions have not been observed on either plant in the field.

Black rot has been found on both wild and cultivated vines of Virginia creeper at both Griffin and Tifton, Georgia. Usually infection is moderate and the leaf spots produced are small. Consequently, the disease is of minor importance and causes little damage even to the appearance of the vines. It has been found on Boston ivy at Griffin and at Atlanta, Georgia, and specimens of diseased leaves have been received from Acworth, Georgia. It is often severe, especially in the spring when nearly all the leaves may be infected and considerable defoliation may result. Since Boston ivy is grown as an ornamental, black rot is important chiefly because of damage to the appearance of the vines.

INOCULATIONS

Inoculations were made on two species of *Parthenocissus* (*P. quinquefolia* and *P. tricuspidata*), the mascadine grape (*Vitis rotundifolia* Michx.), two species of native bunch grape (*V. bourquina* Munson ex Viala and *V. labrusca* Linn.), and the European bunch grape (*V. vinifera* Linn.). The vines to be inoculated were grown in pots in the greenhouse where there was no natural infection. Inoculum consisted of pycnospore suspensions obtained by triturating leaf spots bearing pycnidia collected in the field from *P. tricuspidata, P. quinquefolia, V. rotundifolia,* and *V. labrusca.* In

TABLE 1.—*Results of cross inoculations with Guignardia bidwellii on various suscepts.*

Suscepts	Source of inoculum															
	Parthenocissus tricuspidata				*Parthenocissus quinquefolia*				*Vitis rotundifolia*				*Vitis labrusca*			
	Shoots		Leaves		Shoots		Leaves		Shoots		Leaves		Shoots		Leaves	
	Inoc.[a]		Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Parthenocissus tricuspidata	9	88.9	141	18.4	16	75.0	164	32.3	7	0.0	68	0.0	7	0.0	68	0.0
Parthenocissus quinquefolia	7	100.0	104	41.4	8	100.0	88	68.2	7	0.0	95	0.0	8	0.0	98	0.0
Vitis rotundifolia	9	0.0	61	0.0	8	0.0	50	0.0	2	100.0	14	42.9	3	0.0	30	0.0
Vitis bourquina	12	0.0	112	0.0	5	0.0	33	0.0	4	0.0	22	0.0	2	100.0	8	37.5
Vitis labrusca	5	0.0	28	0.0	3	0.0	14	0.0	4	0.0	14	0.0	3	100.0	18	16.7
Vitis vinifera	7	0.0	34	0.0	4	0.0	34	0.0	1	100.0	8	75.0	1	100.0	9	55.6

[a] Inoc. = inoculated; Inf. = infected.

addition, inoculations were made with suspensions of pycnospores produced in culture on 3 per cent malt agar by ascospore isolates obtained from overwintered leaves of *P. tricuspidata* and *P. quinquefolia*. The inoculum was applied with a pipette to all of the leaves of young, rapidly growing shoots. The inoculated plants were then placed in a moist chamber for 48 hours. Symptoms appeared after an incubation period of two to three weeks. On all suscepts only leaves that were immature at the time of inoculation became infected.

The results of these inoculations are presented in table 1. Inoculum from either Virginia creeper or Boston ivy infected both of these suscepts but no species of grape. Conversely, no infection of Virginia creeper or Boston ivy was obtained with inoculum from either muscadine or bunch grapes. On native grapes, inoculum from muscadine grape produced infection only on muscadine grape; inoculum from bunch grape produced infection only on bunch grape. The introduced European bunch grape, however, was susceptible to infection with inoculum from either muscadine grape or native bunch grape.

TAXONOMY

In a previous report (3) it was shown that *Guignardia bidwellii* occurs on muscadine grapes as a distinct race differing in pathogenicity from *G. bidwellii* on bunch grapes. These two races differed morphologically in size of ascospores and pycnospores. In spite of these differences the fungus on muscadine grapes was given taxonomic rank as a form of *G. bidwellii*. From the results of the inoculations reported here (Table 1) it is apparent that *G. bidwellii* exists in three physiologic races: one pathogenic to native bunch grapes, one to muscadine grapes, and one to *Parthenocissus* species. A consideration of morphology (Table 2) supports the classification of these physiologic races as forms of the one species *G. bidwellii* rather than as distinct species. While the race on muscadine grapes is distinct from the other two races in mean width of ascospores, the mean lengths of ascospores for the three races form an intergrading series, the ascospores of the race on *Parthenocissus* species being intermediate and closing the gap between the other two races. Within the race on *Parthenocissus* species there appears to be a difference in mean length of ascospores produced on the two susceptible species, *P. tricuspidata* and *P. quinquefolia*. The differences in size of pycnospores of the three races are even less than those in size of ascospores; and reference to data published previously (3) shows that the variation among sets of measurements of pycnospores within the same race may be as great as the variation among different races. Although there seems to be a definite tendency toward the production of larger ascospores and pycnspores in the race on muscadine grapes, the differences are too subtle to be of practical use in the separation of these races.

In type of colony produced in culture, the race on *Parthenocissus* species again is intermediate between the race on bunch grapes and the race

on muscadine grapes. On 3 per cent malt agar, the race from bunch grapes produced a white, rapidly growing mycelium that became gray or tan with age. The pycnidia were scattered over the mycelium. In the race from muscadine grapes, the mycelium was black and formed a hemispherical, stromatic mass on which the pycnidia were densely grouped. Growth was never extensive. Colonies of the race from *Parthenocissus* species were similiar to those of the muscadine race in that the mycelium was black and the pycnidia were densely grouped on the surface. The growth was much more extensive, however, forming a flat, wide-spread subiculum on the surface of the agar.

Although the race on *Parthenocissus* species was intermediate between the other two races in morphology and in growth in culture, it showed a greater difference in pathology. While the race on muscadine grapes and

TABLE 2.—*A comparison of spores produced by Guignardia bidwellii on different suscepts*

Suscept	Ascospores		Pycnospores	
	Range *microns*	Mean[a] *microns*	Range *microns*	Mean[b] *microns*
Vitis labrusca	10.6–16.5 × 5.3–8.0	13.15 × 6.55	7.5–10.9 × 5.4–7.5	8.42 × 6.78
Parthenocissus quinquefolia	11.7–16.0 × 5.3–8.0	13.70 × 6.55	7.5–10.2 × 5.4–8.2	8.53 × 6.74
Parthenocissus tricuspidata	10.9–18.4 × 4.8–7.9	14.50 × 6.50	7.1–10.9 × 6.5–8.6	8.46 × 7.30
Vitis rotundifolia	12.6–17.3 × 6.0–9.0	14.90 × 7.10	7.2–13.9 × 5.4–8.2	9.26 × 6.93

[a] 200 spores.
[b] 100 spores.

the race on native bunch grapes have a common host in *Vitis vinifera*, it was impossible to obtain infection of this species with the *Parthenocissus* race.

In view of the intergradations in morphology, it is proposed that the fungi on bunch grapes, muscadine grapes, and *Parthenocissus* species continue to be referred to the single species, *Guignardia bidwellii*, and that within this species three forms, separated upon the basis of pathogenicity, be recognized.

Guignardia bidwellii (*Ell.*) *Viala and Ravaz*

Ascocarps separate, black, spherical, 61–199 μ diam., innate, erumpent and ostiolate at the apex, on overwintered berry mummies and dead leaves; centrum pseudoparenchymatous; asci fasciculate, aparaphysate, cylindrical to clavate, 36–56 × 12–17 μ, short-stipitate, 8-spored; ascus wall thick, composed of two layers; ascospores hyaline, non-septate, ovoid or oblong, straight or inequilateral, rounded at the ends, biseriate, 10.6–18.4 × 4.8–9.0 μ.

Pycnidia black, spherical, 59–196 μ diam., innate, erumpent and ostiolate at the apex; produced on the host during the growing season; on leaf blades amphigenous in circular, reddish-brown, necrotic spots; on stems,

tendrils, peduncles, and petioles in elliptical to elongate, brown to black cankers; on fruits either in berry mummies or in brown to black superficial scabs and cankers; pycnospores hyaline, non-septate, ovoid to oblong, rounded at the ends, 7.1–14.6 × 5.3–9.3 μ.

Spermogonia black, spherical, 45–78 μ diam., innate, erumpent and ostiolate at the apex, produced toward the end of the growing season on berry mummies or dead leaves in association with ascogonial stromata; spermatia hyaline, non-septate, bacilliform, about 2.5 × 1 μ.

On *Vitis* and *Parthenocissus* species.

1. **Guignardia bidwellii** f. **euvitis** f. nov. Pathogenic to American species of Euvitis and to ***V. vinifera.***
2. *Guignardia bidwellii* f. *muscadinii* Luttrell (Phytopath. 36: 913. 1946.) Pathogenic to *Vitis rotundifolia* and *V. vinifera.*
3. **Guignardia bidwellii** f. **parthenocissi** f. nov. Pathogenic to *Parthenocissus* species.

The genetic connection of the perfect and imperfect stages of the forms *euvitis* and *muscadinii* has been demonstrated by previous inoculations (3). The present inoculations with pycnospores from ascospore isolates of the form *parthenocissi* establish the same point for this form.

DISCUSSION

Results of these inoculations show that *Guignardia bidwellii* comprises three physiologic forms: f. *euvitis* pathogenic to native bunch grapes, f. *muscadinii* pathogenic to muscadine grapes, and f. *parthenocissi* pathogenic to *Parthenocissus* species. Infection from cross inoculations with these three forms on the three groups of suscepts could not be obtained. It seems unlikely, therefore, that black rot on either Boston ivy or Virginia creeper may serve as a source of inoculum for the disease on muscadine grapes and bunch grapes.

The recognition of physiologic forms in *Guignardia bidwellii* is not without precedence in the Ascomycetes. Physiologic specialization has been reported in many species of the Erysiphales, Hypocreales, Sphaeriales, and Dothideales (7); and it seems desirable to give these races differing in pathogenicity the taxonomic rank of form. In the Mycosphaerellaceae physiologic specialization has been reported previously by Roark (5) who found that *Mycosphaerella rubi* could be separated into two races, one pathogenic to blackberry, the other to raspberry. To this may be added the present report on *Guignardia bidwellii.* Since species in *Mycosphaerella* and *Guignardia* have frequently been based on host differences, it is probable that a taxonomic revision of these genera would result in a reduction in number of species and the recognition of physiologic forms in many additional species.

SUMMARY

Cross inoculations have shown that *Guignardia bidwellii* (Ell.) Viala and Ravaz, cause of black rot of bunch grapes, muscadine grapes, Virginia

creeper (*Parthenocissus quinquefolia* (L.) Planch.), and Boston ivy (*P. tricuspidata* (Sieb. and Zucc.) Planch.), comprises three physiologic races differing in pathogenicity. Although slight differences in morphology and in growth in culture accompany these differences in pathogenicity, the three races are classified as forms of *G. bidwellii* as follows: *G. bidwellii* f. *euvitis* f. nov., pathogenic to native bunch grapes and (*Vitis labrusca* Linn., *V. bourquina* Munson ex Viala) and to *V. vinifera* Linn.; *G. bidwellii f. muscadinii* Luttrell, pathogenic to muscadine grapes (*V. rotundifolia* Michx.) and to *V. vinifera;* and *G. bidwellii* f. *parthenocissi* f. nov., pathogenic only to *Parthenocissus* spp. Because of physiologic specialization in *G. bidwellii* it seems unlikely that the fungus on Virginia creeper and Boston ivy may serve as a source of inoculum for the black rot disease on either muscadine or bunch grapes.

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STUDIES OF TWO VIRUSES CAUSING MOSAIC DISEASES OF SOYBEAN[1]

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(Accepted for publication April 28, 1948)

INTRODUCTION

The great expansion of soybean (*Soja max* Piper) culture in recent years has led to a large-scale breeding program, and to an increased study of the diseases of this crop. In 1916 Clinton (1) described the symptoms of soybean mosaic; in 1921 Gardner and Kendrick (2) established the virus nature of the disease, and in 1924 they (7) showed that the virus was transmitted from generation to generation through the seed of infected plants. Since that time almost no work has been done on the disease in this country. Heinze and Köhler (4) determined the properties and demonstrated aphid transmission of a virus causing soybean mosaic in Germany. Observations by a number of pathologists indicate that soybean mosiac is present in all major soybean producing areas, and that it causes losses in both quality and quantity of seed.

Certain of the more tolerant varieties of soybean which rarely show symptoms under field conditions have come to be regarded as "resistant" to mosaic. The feeling has arisen that these tolerant varieties maintain their "resistance" as long as they are grown in their areas of adaptation; however, when grown in different regions, they appear to be susceptible. If it were true that a mere change of location would alter the resistance of a group of varieties, plant breeders would be confronted with serious mosaic problems. It is a common practice for crosses to be made between varieties adapted to widely different environments.

Wide differences in mosaic symptoms seen in the field suggested that more than one virus might well be responsible for the mosaic disease of soybean. Some pathologists (8, 10) have shown that certain leguminous viruses are capable, under experimental conditions, of causing systemic infection of soybean. This paper reports the results of an investigation made to clarify a number of these aspects of our knowledge of soybean mosaic.

MATERIALS AND METHODS

Thirty-two virus isolates were obtained from naturally infected, field-grown soybeans in the summer of 1946. These were differentiated by inoculation to soybean, tobacco (*Nicotiana tabacum* L., Havana type No.

[1] Excerpt from a thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of the University of Illinois, 1947.

The author expresses his sincere appreciation to Dr. W. B. Allington and Professor Neil E. Stevens for their interest and guidance in this work.

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38), and garden bean (*Phaseolus vulgaris* L., variety Burpee's Stringless). Fifteen of the isolates were proved by the reaction on tobacco to be the tobacco ring-spot virus commonly found on soybean and recognized as the cause of bud blight. These were discarded.

Fifteen of the isolates were infectious only to soybean. These isolates, which tests indicated were separate isolations of the same virus, produced symptoms agreeing with those described by Clinton (1), and Gardner and Kendrick (2), and the seed from infected plants produced diseased seedlings. This virus will be referred to subsequently as *Soja virus 1* and the disease caused by this virus on soybean as soybean mosaic.

Two of the virus isolates were infectious to garden beans in addition to soybeans. These reacted identically and were considered separate isolations of the same virus. Tests, reported later in this paper, show this virus to be a strain of *Phaseolus virus 2*, the virus causing yellow mosaic of garden bean. This virus will be referred to as *Phaseolus virus 2* and the disease caused on soybean as yellow mosaic.

The soybean variety Bansei was used throughout the investigation as the "standard" variety. The soybean seed used was provided by the U. S. Regional Soybean Laboratory, Urbana, Illinois. Except for the variety Cherokee all seeds were free from mosaic. Other seeds were provided by the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, or were purchased from reputable seedsmen.

Except for some field studies of seed transmission, all experimental work was done in the greenhouse at temperatures which usually varied between 18° C. and 24° C. Supplemental light from 500-watt incandescent bulbs was supplied during the winter period of low intensity. In the design of the experiments and interpretation of results the possibility of accidental insect transmission of the viruses was constantly considered and adequate precautions were taken.

Soybeans were inoculated on both primary leaves when the first trifoliate leaf was less than one-fourth inch long. Carborundum was used in all inoculations. Virus properties were determined according to usual methods, each test being repeated from three to five times.

EXPERIMENTAL RESULTS

Symptomology. On Bansei soybeans the first visible symptom of disease, following mechanical inoculation with *Soja virus 1*, occurs as a yellowish vein-clearing which develops in the minor veins of the developing trifoliate leaves. This symptom is transitory and appears from 6 to 14 days after inoculation. Rugose symptoms usually appear on the third trifoliate leaf formed after inoculation, increase in severity on successive leaves and eventually becomes dark green, puffy areas among the major veins of the leaf (Fig. 1, A). These vesicles, formed by the upward proliferation of the blade, may be scattered or aligned on either side of

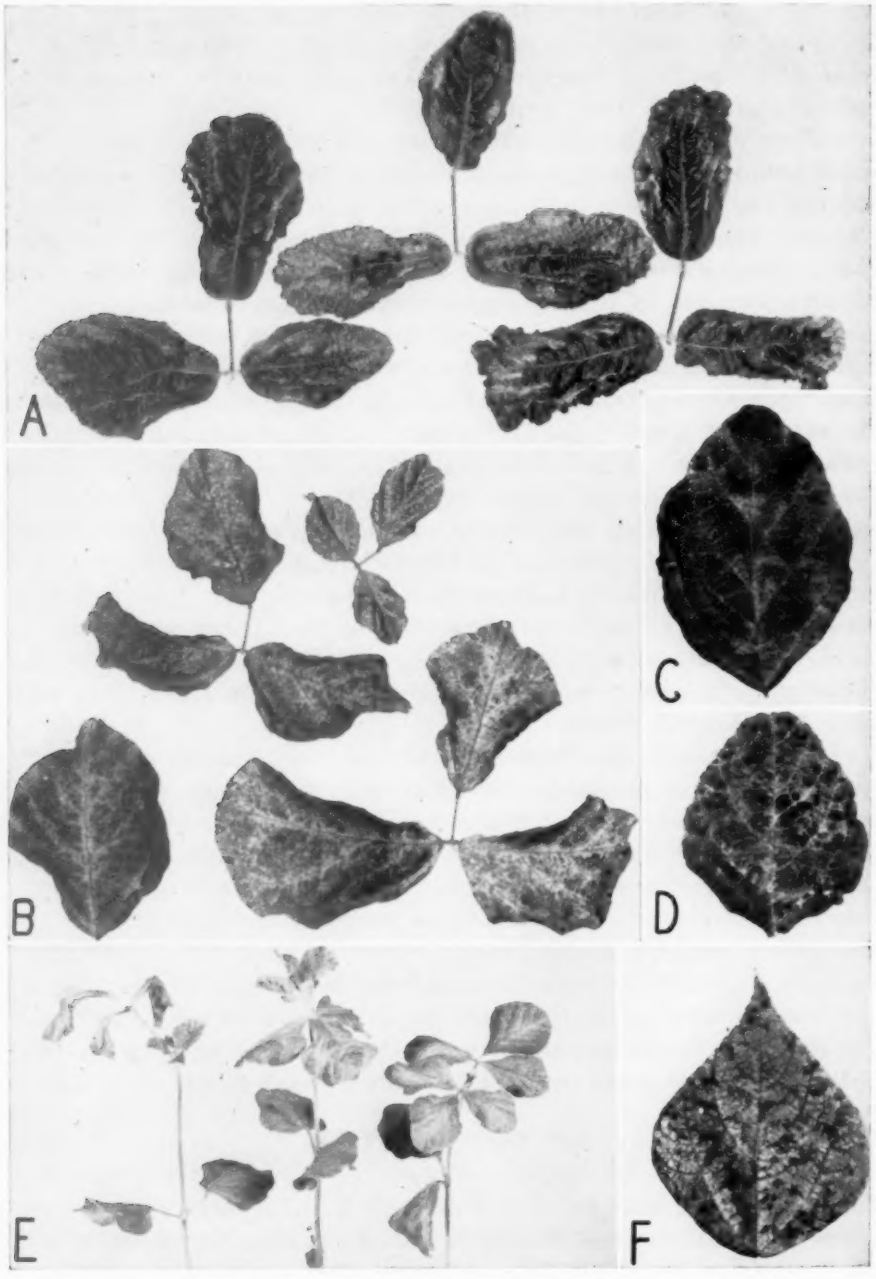

FIG. 1. Symptoms of soybean mosaic on Bansei (A) and Flambeau (B) soybeans. Yellow mosaic symptoms on Bansei (C) and Flambeau (D) soybeans. E. Symptoms produced by seed-borne *Soja virus 1* on Lincoln seedlings. F. Mottling of Burpee's Stringless garden bean leaflet by *Phaseolus virus 2* isolated from soybean.

the major veins. Leaflets may be yellowed among the vesicles and around the margin. Vein-clearing of the matured leaves is common. Leaf margins frequently are curved downward at the sides and upwards at the tip. Leaves become coarse, leathery to the touch, and somewhat brittle at maturity. Diseased plants are stunted and appear to set fewer pods than normal plants. Pods borne on diseased plants are mostly normal; however, some may be curved laterally or twisted and have less conspicuous pubescence. Many pods produced on diseased plants do not bear seed. With the exception of the variety Ogden, the reaction of the tested oil-type varieties is uniform and much less severe than the reaction of Bansei. Leaves of the oil-type varieties are somewhat wrinkled but the conspicuous dark green vesicles characteristic of the disease on Bansei are lacking. Margins of the leaflets tend to be wavy, and bend downward or roll inward in an irregular manner (Fig. 1, B). Occasionally the leaves have a slight yellowish mottle.

The reaction of the Ogden variety to *Soja virus 1* is different from the reactions of the other varieties tested. From 10 to 20 per cent of the plants develop a progressive necrosis which ultimately reaches the terminal bud. The leaves produced on occasional branches are severely dwarfed, mottled, distorted, and dotted with necrotic spots. Since this type of reaction approximates the reaction of soybeans to the tobacco ring-spot virus, inoculations were made from such plants to tobacco to rule out the possibility of accidental tobacco ring-spot virus infection. Tobacco plants were unaffected by the inoculation, and transfers from the inoculated tobacco to Bansei were negative indicating that the virus was not present in masked form in tobacco. Bansei seedlings, inoculated with juice from such Ogden plants, had symptoms typical for *Soja virus 1.*

No marked distinction between vegetable and oil-type soybeans, or between varieties, was observed as a result of infection with *Phaseolus virus 2.* Initial symptoms occur essentially as described for *Soja virus 1;* however, they are less transitory. Successive leaves have a conspicuous yellow mottle as the characteristic symptom (Fig. 1, C and D). The yellow areas may be scattered or occur as indefinite bands along the sides of the major veins. Under certain conditions intense yellow islets appear in the blade. As the leaves mature, rusty necrotic spots develop in the yellowed areas. Diseased plants as a rule are not markedly stunted, and, in the greenhouse, pod set was not appreciably diminished. No symptoms were observed on the pods.

Effect of air temperature on symptom expression. Observations that soybean mosiac is more severe in certain parts of the growing season suggested that the symptomology of the disease is correlated with temperature. Johnson (6) gave some indication of the relationship when he stated ". . . soybean mosaic was completely inhibited at temperatures of 26–28° C." In order to obtain a more complete picture of the relationship a further investigation was made. Three constant temperature cabinets

set at 18.5° C., 24° C., and 29.5° C. were used for this purpose. It was thought that varieties adapted to a growing region with a certain mean temperature might react similarly to each other and differently from varieties adapted to other regions with different mean temperatures. The soybean varieties used to test this possibility included Mandarin and Habaro, northern varieties; Lincoln and strain A4–107–12[3], central types; Louisiana Green and Cherokee, southern varieties. In addition, the vegetable variety Bansei, a mid-season variety in Illinois, was included. Test plants were inoculated and left at usual greenhouse conditions until the initial symptoms of mosaic developed, after which they were transferred to appropriate cabinets. Noninoculated plants of the same age and subjected to the same environment were included in all tests. At least nine plants of each variety were used in each test, and all tests were repeated.

The first two or three leaves formed on seedlings inoculated with *Soja virus 1* were much alike at all temperatures, and were similar to symptoms developing on duplicate plants left in the greenhouse. Subsequent leaves, however, showed striking differences at the various temperatures. This was most spectacular with the variety Bansei, but was evident with all other varieties except Cherokee in which there was only a mild response. Infected Bansei plants grown at 18.5° C. for four weeks were markedly stunted in leaf size and plant height. Leaflets were extremely rugose and were curled upward around the margin. Pubescence on both stem and leaves was extraordinarily apparent. Symptoms on infected Bansei plants at 24° C. were much less severe. Leaflets were considerably distorted, and spotted with raised, dark green swollen areas. Mottling was also apparent at this temperature. Infected Bansei plants grown at 29.5° C. had very mild or no recognizable symptoms (Fig. 2, A). Some leaflets were obscurely mottled, occasionally margins were wavy, but no rugosity or stunting was apparent. The oil-type varieties infected with *Soja virus 1* reacted rather uniformly but did exhibit some varietal differences not correlated with areas of adaptation. Leaflets of Lincoln grown at 18.5° C. were rolled under and inward, misshapen, and considerably wrinkled; however, they lacked the extreme rugosity characteristic of Bansei at this temperature. At 24° C. leaflets were mottled, had wavy margins, but no crinkling of the blade. At 29.5° C. infected plants were essentially symptomless (Fig. 2, B). Habaro, Mandarin, and strain A4–107–12 reacted as did Lincoln. Louisiana Green was more severely dwarfed and distorted than Lincoln at 18.5° C., and the leaflets were mottled at 29.5° C. Cherokee was mildly affected at 18.5° C. and without noticeable symptoms at other temperatures. The varieties adapted to a given region did not react as a group. There was as much variation within the varieties adapted to a certain region as there was between those of different regions. Inoculations to healthy Bansei seedlings from the infected, symptomless plants of all varieties grown at 29.5° C. resulted without exception in the recovery of the virus.

[3] One of the sister strains composited to make the variety Hawkeye.

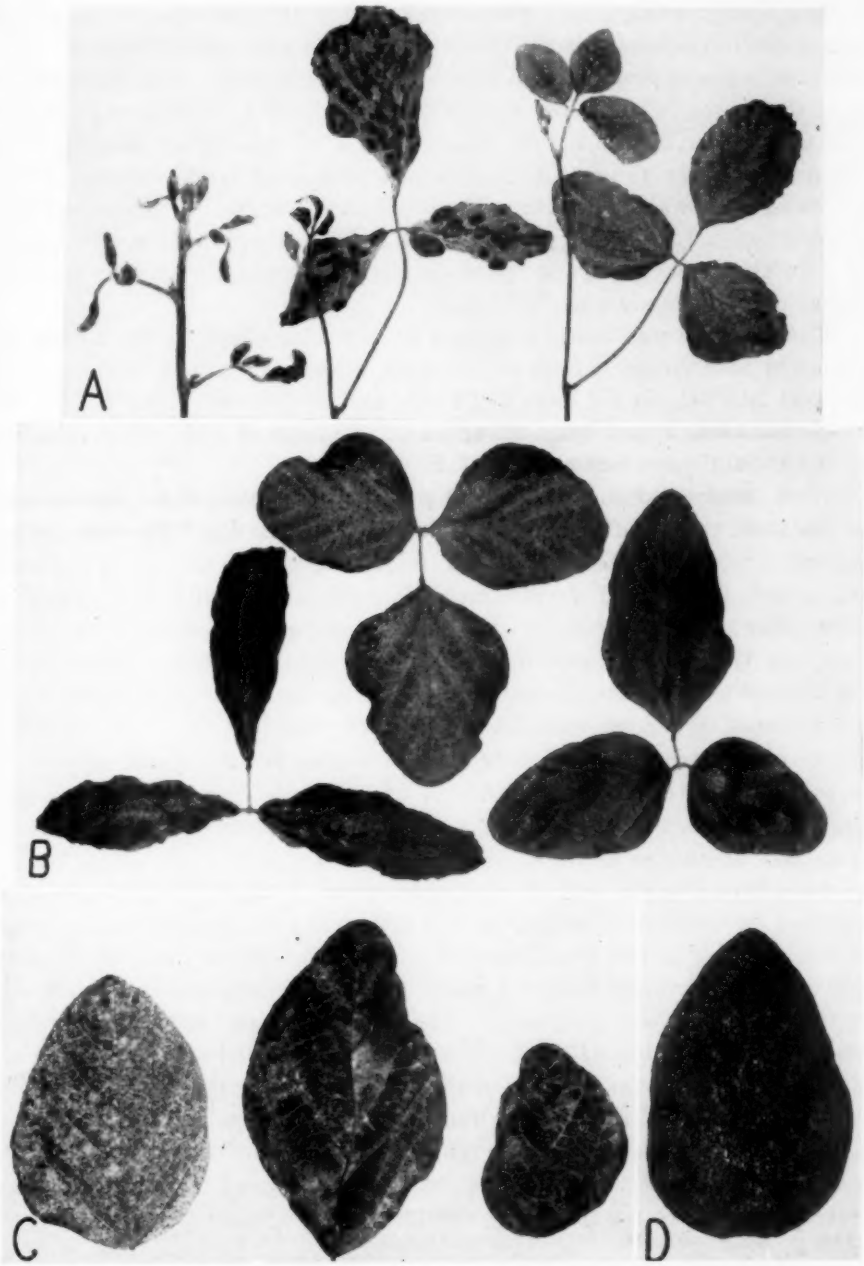

FIG. 2, A–C. Mosaic symptoms on soybeans after 31 days in constant temperature cabinets at (from left to right) 18.5° C., 24° C., and 29.5° C. A. Stem tips of variety Bansei infected with *Soja virus 1*. B. Leaves of Lincoln variety infected with *Soja virus 1*. C. Leaflets of Bansei variety infected with *Phascolus virus 2*. D. Healthy Bansei leaflet.

The symptomology of soybeans infected with *Phaseolus virus 2* was found not to vary conspicuously with either temperature or variety. Yellow mottling was obvious at all temperatures used; however, a slight difference was noted in the type of mottling. At 18.5° C. mottling on Bansei was of a rather diffuse nature, while at 29.5° C. the yellowed areas were fewer and larger (Fig. 2, C). A slight puckering tendency around the yellowed areas was noticeable at the latter temperature. Oil-type varieties reacted similarly to Bansei; the varieties Habaro and Mandarin were noticeable in that rusty, necrotic spots developed abundantly on the matured leaves of plants grown at 18.5° C.

Temperature was found to have a considerable effect on the incubation period of both viruses. *Soja virus 1* symptoms on Bansei appeared in four days at 29.5° C., in six days at 24° C., and in fourteen days at 18.5° C. *Phaseolus virus 2* symptoms appeared in five days at 29.5° C., seven days at 24° C., and twelve days at 18.5° C.

Host range studies. Table 1 presents data showing the species tested as hosts and results of inoculations with *Soja virus 1* and *Phaseolus virus 2*. Attempts were made, in most cases, to recover the viruses from both the inoculated leaves and from leaves formed subsequent to inoculation. *Soja virus 1* did not produce symptoms on any of the species tested; however, the virus was recovered from the symptomless, inoculated leaves of the Burpee's Stringless, Stringless Green Pod, and Stringless Green Refugee varieties of garden beans 23–27 days after inoculation. The virus was not recovered from the trifoliate leaves of these varieties; and it was not recovered from either the inoculated or trifoliate leaves of the Corbett Refugee and U. S. #5 Refugee varieties. The virus was not recovered from any other species tested.

Inoculation of the various species tested as hosts of *Phaseolus virus 2* resulted in systemic mottling in the following: white and yellow sweet clover, crimson clover, broad bean, Alaska and telephone varieties of peas, and all the varieties of garden beans tested. The virus was recovered only from plants showing symptoms. The first symptom observed in garden beans was a drooping of the leaflets at the pulvini; however, this was not obvious in all varieties. Later small halo-like spots appeared in the blades which enlarged and coalesced to form a conspicuous yellow mottling (Fig. 1, F). Mottling became more distinct as the leaves matured, and at maturity leaflets were cupped downward and appeared rather glossy. Affected plants were not markedly stunted and no symptoms were produced on the pods.

Physical properties of the viruses. Thermal inactivation point determinations were carried out by placing 1 ml. of inoculum (sap diluted with an equal volume of distilled water) in thin-walled, small glass tubes and heating for 10 minutes in a water bath. In longevity in vitro tests the inoculum (undiluted sap) was placed in stoppered test tubes and stored

at 18.5° C. in diffuse light. All property tests were repeated at least three times except the longevity in vitro test with *Phaseolus virus 2* which was performed twice.

TABLE 1.—*Results of inoculations with Soja virus 1 and Phaseolus virus 2 with various species of plants*

Species tested	Results of inoculation with			
	Soja virus 1 No. of plants		*Phaseolus virus 2* No. of plants	
	Inoculated	Infected	Inoculated	Infected
Crotolaria spectabilis Roth.	16	0[a]	16	0
Lespedeza striata (Thumb.) H. & A.	13	0[b]	21	0
Lupinus angustifolius L., blue lupine	18	0[a]	12	0
L. luteus L., yellow lupine	10	0[a]	16	0
Medicago sativa L., alfalfa	14	0[b]	29	0
Melilotus alba Desv., white sweet clover	14	0[b]	20	12
M. officinalis (L.) Lam., yellow sweet clover	14	0[b]	10	4
Phaseolus aureus Roxb., mung bean	15	0	15	0
P. coccineus L. scarlet runner bean	7	0	15	0
P. lunatus L., civet bean, Henderson Bush variety	4	0[a]	14	0
P. mungo L., urd bean	15	0	15	0
P. vulgaris L., garden bean				
Asgrow Black Valentine variety	.....	.....	16	14
Burpee's Stringless variety	98	0[c]	20	18
Corbett Refugee variety	5	0	20	8
Full Measure variety	.....	.....	19	12
Improved Golden Wax variety	.....	.....	11	10
Red Kidney variety	.....	.....	12	8
Stringless Green Pod variety	9	0[c]	.....	.....
Stringless Green Refugee variety	13	0[c]	20	17
Stringless Red Valentine variety	.....	.....	14	13
U. S. No. 5 Refugee variety	4	0	20	12
Pisum sativum L., pea,				
Alaska variety	.....	.....	16	13
Telephone variety	.....	.....	14	11
Trifolium incarnatum L., crimson clover	14	0[a]	14	13
Vicia faba L., broad bean	.....	.....	6	6
Vigna sesquipedalis (L.) Fruwirth, asparagus bean	15	0	15	0
V. sinensis (L.) Endl., cowpea				
Victor variety	15	0	15	0
Whippoorwill variety	15	0	15	0
Beta vulgaris L., beet, Detroit Dark Red variety	17	0	27	0
Lycopersicon esculentum Mill., tomato				
Early Baltimore variety	11	0	4	0
Nicotiana tabacum L., tobacco				
Havanna type No. 38	39	0	12	0

[a] No attempt made to recover *Soja virus 1*.

[b] No attempt made to recover *Soja virus 1* from inoculated leaves; however, recovery attempts from tops were negative.

[c] *Soja virus 1* recovered from symptomless, inoculated leaves 23–27 days after inoculation.

Soja virus 1 had a thermal inactivation point lying between 64° C. and 66° C. The virus was still infectious after four days aging in vitro, but was non-infectious after five days. The results of five attempts to establish the tolerance to dilution varied so widely that no definite value was attained.

The thermal inactivation point of *Phaseolus virus 2* was determined to be between 54° C. and 56° C. The virus had a longevity in vitro of three to four days. The dilution end-point varied so widely in five tests that no definite value was obtained.

Seed transmission studies. It has been demonstrated that soybean mosiac is seed transmitted (4, 7, 8). The discovery that *Phaseolus virus 2* causes a field disease of soybeans made the question of whether it is seed transmitted of practical importance. In order to answer this question, and to confirm seed transmission of *Soja virus 1*, numerous naturally infected plants were tagged in the field in the summer of 1946 and the seed from them collected individually. In addition some seed was collected from inoculated plants grown in the greenhouse. This seed was grown in the greenhouse the following winter and in the field in the summer of 1947. Seedlings grown in the greenhouse were observed until flower buds were formed, final readings in the field were made when the plants were approximately 15 inches tall.

Seed from plants[4] tagged in the field as having soybean mosaic produced a relatively high percentage of diseased seedlings, varying from 2 to 75 per cent according to soybean type. Of 1,993 seedlings grown from such seed, 646, or 32 per cent, were diseased. Seed collected from the variety Lincoln, inoculated and grown in the greenhouse, produced 364 seedlings, 15 per cent of which were diseased. Symptoms produced on seedlings by seed-borne *Soja virus 1* were usually observed on the primary leaves. Such leaves had wavy margins and the blade was somewhat crinkled or slightly mottled. The first trifoliate leaf usually showed symptoms characteristic of soybean mosaic on that variety (Fig. 1, E). Seed from several plants tagged in the field as having soybean mosiac failed to produce diseased seedlings.

A total of 362 seedlings were grown from the seed of soybeans inoculated with *Phaseolus virus 2*. None of these were diseased. Seed from plants, mostly strain A4–107–12, tagged in the field produced 6,323 seedlings; none of these had yellow mosaic. Some seed was collected from garden beans inoculated with *Phaseolus virus 2*. The 107 seedlings grown from this seed were all healthy.

Insect transmission. The pea aphid, *Macrosiphum pisi* Kalt.,[5] the peach aphid, *Myzus persicae* Sulzer, and *Thrips tabaci* Lindeman were used in attempts to transmit the viruses by insects. These were cultured on healthy plants of alfalfa, tobacco, and soybean respectively. Aphids were fed on diseased plant parts placed in large diameter glass tubes covered at the ends with several thicknesses of cheesecloth. After feeding 24 to 30 hours on the diseased tissue, the aphids were transferred with usual precautions to healthy seedlings which were enclosed immediately in

[4] Mostly soybean introductions of United States Department of Agriculture.

[5] Insects were identified by Dr. H. H. Ross, Systematic Entomologist, Illinois State Natural History Survey, Urbana, Illinois.

bell-jars. Under these conditions aphids were dead after five to six days. Similar technique was followed with thrips except that they were collected from diseased soybeans and transferred directly to healthy seedlings in bell-jars.

Both the pea aphid and the peach aphid transmitted *Soja virus 1* to healthy seedlings. In the two tests resulting negatively with the former species only four aphids were transferred to each healthy seedling; ten or more aphids were transferred to each seedling in the six tests resulting positively. Both tests with the peach aphid resulted in virus transmission. Aphids became viruliferous feeding on infected stems, old or young leaves, and stem tips. Four attempts to obtain transmission with thrips were negative. From 50 to 100 thrips were transferred to each healthy seedling. All check plants remained healthy. The incubation time, initial symptoms, and course of development of the disease following infections by insect-transmitted virus was the same as that following mechanical inoculation.

Successful transmission of *Phaseolus virus 2* was obtained only once in nine separate tests with *Macrosiphum pisi*. In this case aphids were fed on diseased garden bean leaves and transferred to garden bean seedlings. Attempts to transmit this virus from soybean and garden bean to soybean were negative. Transmission of this virus with other insects was not attempted.

DISCUSSION

The experimental evidence confirms the observation that temperature plays a large role in the symptomology of soybean mosaic. This also confirms and extends the work of Johnson (6) who reported only that high temperature suppressed symptoms. Tests to determine varietal resistance or immunity should be made at temperatures suitable for symptom expression. It is also clear that the reaction of a variety to *Soja virus 1* is not correlated with the area to which it is adapted; rather the variety is the unit in which the reaction is determined. If there is a danger of creating a soybean variety especially susceptible to soybean mosaic by making crosses between varieties adapted to wide environments the dangerous entity is within the individual varieties used in crossing.

The values obtained for the thermal inactivation point and longevity in vitro of *Soja virus 1* are similar to those obtained by Heinze and Köhler (4). Pierce (8) reported a thermal inactivation point of 56°–58° C. for a seed-transmitted virus causing soybean mosaic; this differs so much from the writer's results that it cannot be presumed the same virus is involved. *Soja virus 1* induced systemic symptoms only on soybeans; this confirms the work of Gardner and Kendrick (2), Pierce (8), and Heinze and Köhler (4). The latter authors reported that the virus produced symptoms and could be recovered from the inoculated leaves of certain varieties of *Phaseolus vulgaris;* the writer was able to recover the virus from the inoculated primary leaves of certain varieties of this species but no symptoms were observed on the inoculated leaves.

The suggestion of some pathologists (5) that soybean "mosaic" is caused by more than one virus is substantiated by this investigation. A second virus was collected from soybean which differed from *Soja virus 1* in symptomology, physical properties, host range, and seed transmission; it closely resembled *Phaseolus virus 2* in physical properties, host range, symptomology, and lack of seed transmission in garden bean. Pierce (8), who found soybean susceptible to *Phaseolus virus 2*, reported collecting the virus from soybeans in the field; however, no description of the disease on soybean was recorded. The virus collected from soybean by the writer differs from *Phaseolus virus 2*, as described by Pierce (8) and Zaumeyer and Wade (10), by not being infectious to mung bean and lespedeza, and by having a thermal inactivation point of 54°–56° C. as compared with 56°–58° C. for *Phaseolus virus 2*. Because of these differences it appears best to regard the virus isolated from soybeans as a strain of *Phaseolus virus 2*.

It has been demonstrated that soybean mosaic spreads in the field (3, 4, 7). Kendrick and Gardner (2) were unable to obtain transmission with the tarnished plant bug and leafhoppers. Heinze and Köhler (4) found that, in Germany, eight species of aphids, among them the peach aphid, were capable of transmitting *Soja virus 1*. Transmission of the virus was accomplished in this investigation by the peach aphid and the pea aphid. While the pea aphid has been reported a vector of other legume viruses, this is the first report that it also serves, under experimental conditions, as a vector of *Soja virus 1*. Soybeans were not a favored host of aphids under the conditions prevalent in this study. In the greenhouse aphids rarely increased in numbers or persisted for an appreciable length of time on soybeans. It is believed that aphids may be responsible in part for spread of soybean mosaic in the field. Zaumeyer and Kearns (9) found a parallel situation in their studies on the transmission of common mosaic of bean (*Phaseolus virus 1*). They obtained transmission with a number of aphid species, but found that beans were not a favored host of these insects. They believed that aphids in searching for a more favorable host might feed in the interim on beans, thus spreading the disease. It is likely that under field conditions other insects are capable of transmitting the viruses causing the mosaic diseases of soybean.

SUMMARY

A study was made of two viruses causing mosaic diseases of soybean. The symptomology of soybean mosaic, caused by *Soja virus 1*, and of yellow mosaic, caused by a strain of *Phaseolus virus 2*, was described.

Air temperature had a considerable effect on the symptomology of soybean mosaic, symptoms being severe at 18.5° C., and largely masked at 29.5° C. Soybean varieties were found not to react as groups according to areas of adaptation, but rather as individual varieties. *Soja virus 1* produced systemic infection only on soybeans; however, it was recovered from

the symptomless, inoculated, primary leaves of certain varieties of garden beans. The thermal inactivation point of *Soja virus 1* was from 64° to 66° C.; longevity in vitro 4–5 days. The virus was transmitted through the seed of both inoculated and naturally infected plants. The pea aphid and the peach aphid transmitted the virus from infected to healthy plants; transmission tests with *Thrips tabaci* were negative.

Air temperature did not markedly affect symptom expression of yellow mosaic. All soybean varieties tested reacted similarly to *Phaseolus virus 2*. Besides soybeans, this virus induced systemic mottling on *Phaseolus vulgaris, Vicia faba, Trifolium incarnatum, Pisum sativum, Melilotus alba,* and *M. officinalis*. The thermal inactivation point of this virus was from 54° to 56° C.; longevity in vitro 3–4 days. The virus was not seed transmitted in 6,685 seedlings grown from the seed of inoculated and naturally infected soybeans, or in 107 seedlings from seed of inoculated garden beans.

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COLLAR INJURY OF APPLE TREES ASSOCIATED WITH WATERLOGGED SOIL

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When collar injury appears on apple trees the disturbance is usually attributed either to winter injury or to a pathogen such as *Phytophthora*. Winter injury, however, is often complicated by the condition of poor drainage or a waterlogged soil, thus making it difficult to separate cold injury from that due to poor aeration. The collar injury here described occurred under conditions that apparently precluded winter injury as a contributing factor, and for that reason deserves attention. The observations on collar injury to apple trees herein reported were made during the summer of 1945 on a block of trees at the Plant Industry Station, Beltsville, Md., containing 760 5-year-old trees.

The trees, originally a part of a rootstock experiment, were being used in inoculation tests with *Phytophthora cactorum* (L. and C.) Schroet. As a preliminary treatment prior to inoculation, the area where the trees were growing was given several applications of water (totaling approximately 10 inches) during the month of May. The apparent harmful effects of this irrigation plus the unusually heavy rainfall of 25.1 inches during May, June, and July are reported in this paper.

Inoculation with *Phytophthora cactorum* was attempted by removing the soil from the collar and inserting the inoculum into the root tissue. This was done on 29 trees on May 28, on 161 trees on June 7, and on 40 trees on July 6. The remainder of the trees in the block were left uninoculated as checks. When the soil was removed in preparation for the July 6 inoculation 2 of the 40 trees showed initial stages of collar injury below the soil line. These two trees had not been disturbed by any previous treatment except irrigation. They were not inoculated, but were kept for further observation of the development of the collar injury that was already starting. The progress of the development of the lesions on these 2 trees was studied during the summer.

The lesions on the 2 trees mentioned above were 30 and 40 mm. in diameter, respectively. The spots were brown and water-soaked and their condition indicated that they were of very recent origin.

On August 22 these two trees were dug up and examined and photographed (Fig. 1). By this time the dead area had extended at least half way around one tree and had girdled the other. It had extended downward as far as the branch roots, a distance of 15 cm., but had extended upward very little farther than when the first observation was made in July. The

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dead area was water-soaked and in places the bark was gelatinous and slimy Demarcation between the living and the dead tissues was usually very sharp Thus the killing started slightly below the soil line and advanced laterally

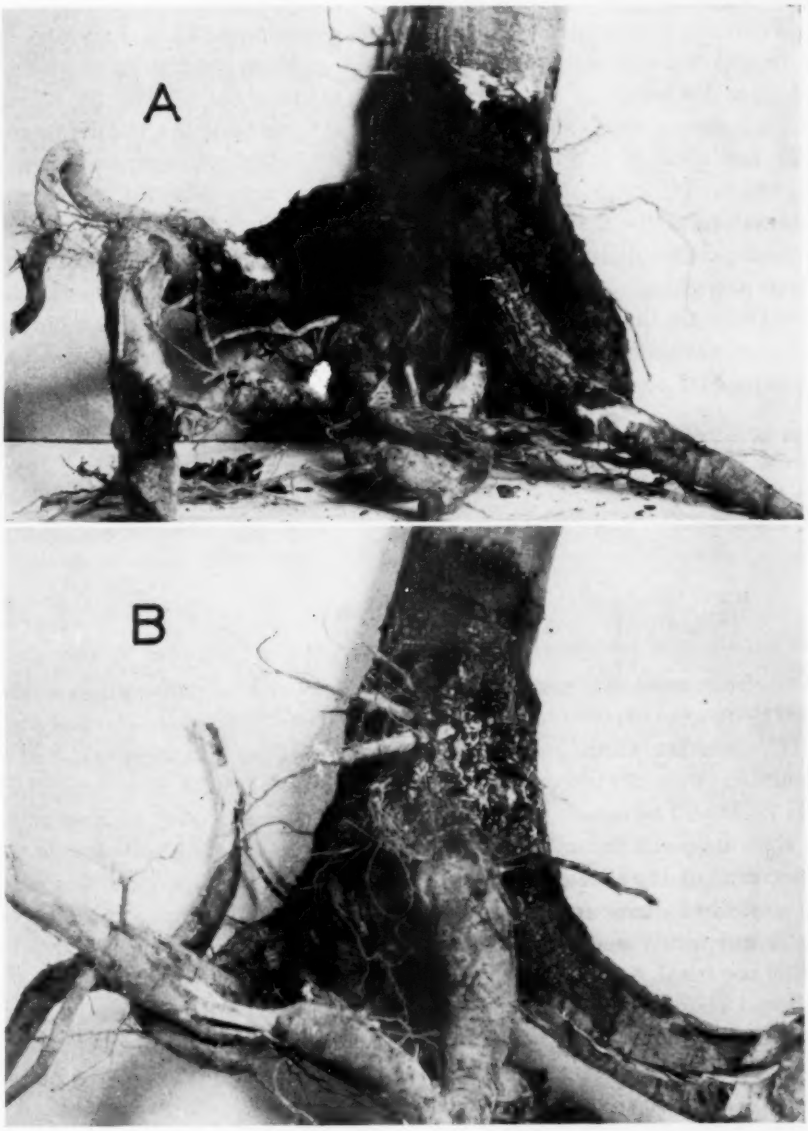

FIG. 1, A. The extensive dead area was a lesion only 40 mm. in diameter on July 6. When photographed on August 23 the tree was completely girdled. B. A companion tree with one side still alive. The enlarged lenticels are characteristic of trees growing in a waterlogged soil. The bark in the upper part of the picture is discolored but not dead.

and downward rather than upward. At this time the tops of the trees having this collar injury showed no abnormal symptoms, which is another indication that the below-ground dying of the bark was very recent and very rapid.

In general, the killing was uniform throughout the affected bark area. However, one could sometimes see islands of live tissue surrounded by dead tissue. Such islands of living tissue, according to the author's experience, do not occur in lesions caused by an apple root pathogen.

When the first evidence of this disturbance appeared in July and while the lesions were relatively small, thorough culture studies were made, but with negative results. Such studies were made again in August, when the two trees were dug up and photographed. At this time the temperature and media particularly favorable for the growth of *Phytophthora* were provided (2), but no evidence of a pathogenic organism was obtained. Microscopic examination at the margin of the dead and healthy tissue did not show any mycelium. The distinctive character of the lesion as well as the failure to isolate a pathogen and the failure to observe mycelium in newly killed tissue indicates that the collar lesions in question were not due to a pathogen.

In a short time after the injury was noted on the two trees similar lesions were appearing not only on trees used in the inoculation studies but also in

TABLE 1.—*Progressive collar injury to apple trees*

Number of trees examined	Percentage of trees having collar injury			
	May 28	June 7	July 6	November 1
39	0	.....	.....	67
161	.....	0	.....	57
38	.....	.....	5	40

the alternate rows that were used as checks for the *Phytophthora* inoculation. The lesions were very irregular in shape and the killing was extremely rapid.

In November a final examination was made of the 238 trees that had been inoculated with *Phytophthora cactorum*. The results are presented in table 1. It will be noted that there was no evidence of injury in May or June, but that incipient injury was showing in July. This table shows that over 50 per cent of the inoculated trees had developed a collar injury typical of that described above for the two trees first observed to have this injury on July 6 and which consequently were not used in the inoculation test. These lesions occurred promiscuously around the collar.

Small lesions developed on a few of the trees (10 per cent) at points where inoculations were made. These lesions were definite in outline but were only 5 to 10 mm. in diameter by November. Reisolation of the pathogen from these small areas was not attempted.

The data in table 1 relate only to trees on which no lesions were found early in the summer, at the time they were inoculated. Examinations were also made in the fall on uninoculated trees that had not been examined earlier in the season. They showed the presence of collar injury at this time, and enough were examined to indicate that the disturbance occurred over the whole block of trees. Observations made on trees that were dead the

following year indicated that collar injury was distributed over the whole block and included both the trees inoculated with *Phytophthora* and the checks for this inoculation test.

The literature contains very little information about collar water injury to pomaceous fruit trees. The few reports that have been made on water injury to fruit trees have been concerned with stone fruits [Fikry (3), Howard (4)], with apple orchards flooded during the winter months [Dorsey and Ruth (1)], and with flooded pear trees [Kienholz (5)]. Pear trees are generally considered more tolerant of a waterlogged soil than apple trees.

SUMMARY

Five-year-old apple trees growing in a heavy soil and having had wet conditions during the summer developed a collar injury during the summer months without the complicating effect of cold injury.

Very small lesions, measuring as much as 10 mm. in diameter, developed on some of the trees inoculated with *Phytophthora cactorum*. Such lesions rarely formed and did not develop further.

The extensive collar injury here described was apparently the result of waterlogged soil.

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A STUDY OF THE FUNGICIDAL ACTION OF 8-QUINOLINOL AND SOME OF ITS DERIVATIVES[1]

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INTRODUCTION

The use of 8-quinolinol and some of its derivatives as fungicides has been mentioned at various times in plant science literature (2, 4, 5, 7, 9, 12, 17, 18, 20, 21, 23, and 24). The 8-quinolinols have been recognized for twenty years or more as having bactericidal properties, but their possibilities as commercial fungicides and plant protectants apparently were not recognized until recently. The cost of the material has been and still is a matter of extreme importance in developing these possibilities. However, Powell (20) has shown that the copper salt of 8-quinolinol has real promise of becoming an important material in the control of scab and blotch of apples. Further field tests at the Illinois Experiment Station have confirmed these first results and it was found that the material also has remarkable properties in the control of bitter rot of apples. The zinc salt showed some promise as a possible protectant of peaches against brown rot.

Because of the large number of possible derivatives of 8-quinolinol, this series lends itself very well to a study of the fungicidal action of the group and the change of toxicity resulting from the substitution of various elements in the parent compound. This paper is an initiation of such a study based on 8-quinolinol and eleven of its derivatives (Table 1).

MATERIALS AND METHODS

The 8-quinolinol and its derivatives were prepared by the Monsanto Chemical Company for experimental use and with the exception of 8-quinolinol sulfate, they were the highest grade obtainable. The 8-quinolinol sulfate was an 8 per cent solution and in a crude form. The ferric dimethyldithiocarbamate used was Fermate produced by E. I. Du Pont de Nemours Company. The chemicals used in making the Bordeaux mixture were Baker and Adamson chemicals produced by General Chemical Company.

Most of these compounds were very insoluble in water. In order to suspend them in water they were made wet with ethyl alcohol and then brought to volume with water. In the case of 8-quinolinol which was

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found to be soluble in ethyl alcohol, the material was dissolved in alcohol and then made to volume with water. It remained in solution after the water was added. Since 8-quinolinol sulfate was water soluble, no alcohol was used in preparing the various dilutions of this material. The maximum amount of alcohol was 4 per cent in the dilutions of 1000 parts per million, 0.4 per cent in 100 parts per million, and a proportional amount in other dilutions.

The molecular structure of 8-quinolinol and representatives of the various types of derivatives included in this study are shown in figure 1. The 5, 7 substitution products simply have the H replaced by Cl, Br, or NO_2 in the 5 and 7 positions of the quinolinol ring or rings.

All glassware was cleaned in perchlorate-sulfuric acid cleaning solu-

TABLE 1.—*List of compounds tested, the abbreviations used in the graphs, and the empirical formula of each compound*

Compound	Abbreviation used	Empirical formula
8-quinolinol	8-Q	C_9H_7ON
8-quinolinol sulfate	8-Q SO_4	$(C_9H_7ON)_2H_2SO_4$
Copper 8-quinolinolate	Cu-8	$(C_9H_6ON)_2Cu$
Magnesium 8-quinolinolate	Mg-8	$(C_9H_6ON)_2Mg$
Manganese 8-quinolinolate	Mn-8	$(C_9H_6ON)_2Mn$
Iron 8-quinolinolate	Fe-8	$(C_9H_6ON)_2Fe$
Zinc 8-quinolinolate	Zn-8	$(C_9H_6ON)_2Zn$
5,7-di-bromo 8-quinolinol	di Br	$C_9H_5ONBr_2$
5,7-di-chloro 8-quinolinol	di Cl	$C_9H_5ONCl_2$
5,7-di-nitro 8-quinolinol	di NO_2	$C_9H_5O_5N_3$
Copper 5,7-di-bromo 8-quinolinolate	Cu di Br	$(C_9H_4ONBr_2)_2Cu$
Copper 5,7-di-chloro 8-quinolinolate	Cu di Cl	$(C_9H_4ONCl_2)_2Cu$
Bordeaux mixture	B. M.	
Ferric dimethyldithiocarbamate	Fermate	$(C_3H_6NS_2)_3Fe$

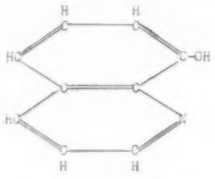
tion as suggested by Morton and Czarnetzky (19) and thoroughly rinsed in tap water and in distilled water.

Two different methods of evaluating fungicides were used. They were the settling tower method and the spore-dilution-drop method as outlined by The American Phytopathological Society Committee on Standardization of Fungicidal Tests (1) except that it was impossible to obtain a water seal since the only moist chambers available had knobs on the tops and could not be inverted. However, the chambers were kept in an air-conditioned room with a temperature of 26° C. $\pm$ 1° and a relative humidity of 50 per cent $\pm$ 2 per cent so that this lack of water seal should be unimportant under these conditions. Because of the possible effect resulting from the volatilization of these compounds, the chambers and slides were removed to the air-conditioned room after spraying and before the spore-drops were added so that the spores were never in the room in which the spraying was done.

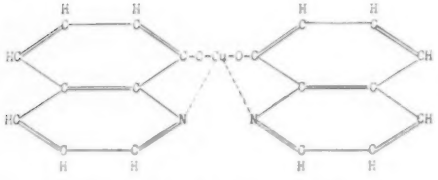
The culture of *Stemphylium sarcinaeforme* (Cav.) Wilts. was obtained from Dr. S. E. A. McCallan of the Boyce Thompson Institute under the

name *Macrosporium sarcinaeforme*, but this was later changed by McCallan (13).

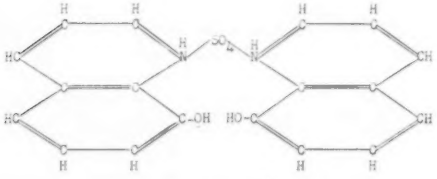
Each treatment was applied to four slides with three drops on each slide. These slides were incubated in one moist chamber together with one untreated slide which was included in order that the volatile effect of the compound could be manifested. One chamber containing four untreated slides was used as a daily check on the normal germination of the spores with no fungicide present. The spores were counted after 18–20 hours' incubation at 26° C.



A. 8-QUINOLINOL



B. Metal salt of 8-QUINOLINOL



C. 8-QUINOLINOL SULFATE

FIG. 1. Structural formulae of representative types of 8-quinolinol compounds.

The method used for determining whether the action of these compounds, which at some point permitted no germination, was fungicidal or fungistatic was similar to that outlined by McCallan and Wellman (14). The compounds which were not completely water soluble were suspended in a flask, stoppered, and shaken for 20 hours on a reciprocal shaker with a $2\frac{1}{2}$-inch stroke at 100 cycles per minute. This suspension was then centrifuged at 870 times gravity for 5 minutes and the supernatant liquid was decanted and used in the same way as the completely water soluble materials. Spores were introduced into these solutions and tests were made by the spore-dilution-drop method to make certain that the solutions were concentrated enough to permit no germination. The remainder of the spore suspension was allowed to remain in a test tube. If no germination occurred, then the suspension was centrifuged and the spores were washed three

times with distilled water. The spores were then brought back to the original volume with distilled water and tested again by the spore-dilution-drop method. Lack of germinated spores at this point indicated that the material was fungicidal; whereas, the presence of germinated spores indicated that the material was fungistatic.

RESULTS

The results of the tests by the settling tower method are represented in figures 2 and 3. The values are an average of 24 counts of 50 spores so that each value is actually the percentage germination based on 1200 spores. These graphs are plotted on logarithmic-probability graph paper.

The results of the tests by the spore-dilution-drop method are presented in figures 4 and 5 in the same manner as figures 2 and 3 except that the concentrations are in parts per million as prepared before the drops were put on the slides.

All of the compounds were tested over essentially the same range of concentrations from 0.5 to 1000 parts per million and from 0.01 to 35 micrograms per square centimeter. Higher concentrations were impractical because of the difficulty of distinguishing the spores from the insoluble material on the slides.

In the fungicidal versus fungistatic tests, it was found that 8-quinolinol, the copper salt, the halogenated derivatives and their copper salts, and 8-quinolinol sulfate were fungicidal under the conditions of these tests. This is not in agreement with the findings of McCallan and Wellman (14) on 8-quinolinol sulfate. However, since the 8-quinolinol sulfate used in these tests was in a crude form, it is possible that other substances may have influenced these results.

The compounds showing vapor toxicity were 8-quinolinol sulfate, 8-quinolinol, the magnesium salt, the manganese salt, the zinc salt, the dichloro derivative, and ferric dimethyldithiocarbamate. This effect was observed only in a qualitative manner since a quantitative method of measuring vapor toxicity is beyond the scope of this study. These results are in agreement with those of Lebduska and Pidra (11). They reported that the vapors of 8-quinolinol inhibited the growth of *Staphylococcus aureus* and *Bacillus coli* in Petri dishes containing one gram of the compound and held for 24 hours.

DISCUSSION

The data presented do not seem to follow the usual pattern of toxicity reactions. All the metal salts of 8-quinolinol (except the copper salt) generally reduced germination with an increase in concentration only to a certain point (figs. 2 and 3). This was then followed by an increase in germination as the concentration was increased further. The same tendency was observed for these compounds in the results represented in figure 4. However, the magnesium salt reversed itself after the concentration reached 180 parts per million and a further increase in concentration resulted in a

decrease in germination. The 8-quinolinol and 8-quinolinol sulfate followed this same trend in the spore-dilution-drop method but not in the settling tower method. McCallan, *et al.* (15) have reported that certain inorganic compounds exhibit a departure from a linear dosage-response curve. Dimond *et al.* (3) also reported a dosage-response curve that was not a straight line for laboratory tests on tetramethyl thiuramdisulfide. However, Horsfall (8) implies that when such trends are observed, one should first examine his methods carefully. In the case of the data presented the same tendency was observed in each of duplicate tests employing two different methods and with each test conducted on different days. The fact that repeated tests in which Bordeaux mixture and the copper salt were used gave very consistent normal results further reduces the probability of these trends being the result of some discrepancy in the methods employed.

Limited field observations have shown a similar trend in the action of the zinc salt when sprayed on peaches as a protectant against brown rot. One quarter pound per 100 gal. of water was more effective than one half pound, which in turn was more effective than one pound in controlling the disease. None of the theories at present seem to adequately explain this type of reaction. It may be that the particles formed larger aggregates as the concentration was increased, and, as a result, there may have been less surface exposed for solution and subsequent reaction with the spores to reduce germination. Gortner (6, p. 186) has presented a table showing the tremendous decrease in surface area for a specific weight of a substance as the particle size is increased. These materials are only very slightly soluble in water and it is possible that with a decrease in exposed surface area through aggregation not so much material would be dissolved in concentrated suspensions and thus brought into reaction with the spore as in the lower concentrations. Another possibility is that suggested by Dimond *et al.* (3). They pointed out the possibility of a change in reaction phase of a material by dissociation or association so that its toxicity is markedly different from the original molecule. If this is true then the toxicity curve should be expected to follow the association-dissociation curve of the same compound.

A third possible factor that has been suggested as having some importance in this reaction is the permeability of the spore at various concentrations of the salts tested. Jacobs (10) cites the work of Osterhout showing that certain compounds may cause a definite decrease in permeability of plant tissue at low concentrations but that an increase in concentration may then cause an increase in permeability to the point where death of the tissue would be expected. If reduction in germination is proportional to the increase of permeability, then the findings of Osterhout would not explain the reactions reported here. This does not, however, eliminate the possible importance of permeability since the 8-quinolinols may affect permeability in a different manner than the compounds used by Osterhout.

The fact that the compounds showing vapor toxicity are the ones pro-

ducing the two-peaked dosage-response curve may be a significant factor in explaining these reactions. Further investigation is needed to show if this connection is significant and the possible relationship if it is significant.

The halogenated derivatives of 8-quinolinol as shown in figures 2, 3, 4, and 5 produced the same general type of reaction as Bordeaux mixture, ferric dimethyldithiocarbamate, and copper 8-quinolinolate (with the exception of the two di-bromo derivatives) by the settling tower method. Copper 5, 7-dibromo 8-quinolinolate did not reduce germination to less than 60 per cent. The halogenated derivatives were much less effective than 8-quinolinol or copper 8-quinolinolate in reducing the germination of spores. Conversion of parts per million to molar concentration failed to eliminate this difference in effect. Thus it is apparent that the substitution of chlorine and bromine for hydrogen in the 5 and 7 positions definitely decreased the ability of the compounds to reduce spore germination of *Stemphylium sarcinaeforme*. The same was true of the 5, 7-di-nitro 8-quinolinol. There was no reduction in germination by this compound at any concentration tested.

This group of compounds responds in a manner different from the phenols as reported by Woodward *et al.* (22) when halogen atoms were substituted into these compounds. They found that the toxicity to the test organisms employed was multiplied several times by such substitutions into the phenols.

The loss of toxicity of 8-quinolinol when substitutions of the nitro group are made at the 5 and 7 positions suggests that toxicity may be the result of a reaction of 8-quinolinol with certain nitrogenous materials vital to the organism. If this reaction has occurred before the compound comes in contact with the organism, such as in the di-nitro derivative, no toxicity would be evident. On the other hand, the substitution of nitro groups or halogens may cause a shift in the internal structure of the molecule in some manner so as to change its reaction.

Copper 8-quinolinolate was found to be considerably more effective than any other material tested by the spore-dilution-drop method. The 8-quinolinol sulfate was slightly more effective in the tests by the settling tower method, but even by this method the concentration of complete inhibition of germination was the same for both materials. Either material was more effective than Bordeaux mixture. The difference is even greater than is indicated in figure 2 because of the fact that the concentration of Bordeaux mixture is based on the deposit of copper only; whereas, the concentration of the other two compounds is based on the deposit of the total material. On the basis of parts per million of copper, the concentration of the copper 8-quinolinolate which will permit no germination is only 1.4, and the concentration had to be reduced to 0.17 parts per million to permit 100 per cent germination.

McCallan and Wilcoxon (16) in comparing several copper fungicides with Bordeaux mixture concluded that fifty years of experimentation with

copper compounds have yielded none equal to Bordeaux mixture. They also stated that apparently no copper compound has yet been developed which has combined high fungicidal values with low phytocidal properties.

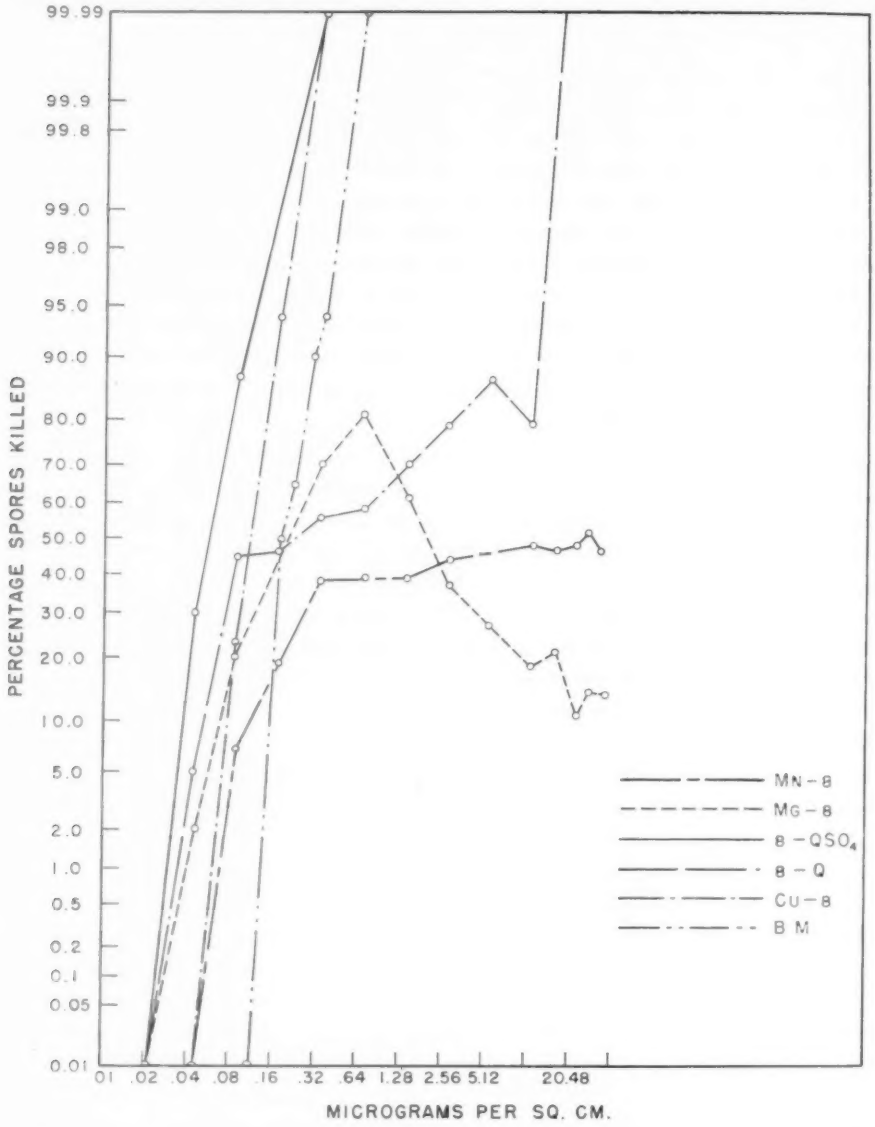


FIG. 2. Dosage-response curve of *Stemphylium sarcinaeforme* by the settling tower method.

It is possible that copper 8-quinolinolate may eventually compare favorably with Bordeaux mixture as a fungicide. In these laboratory studies it has shown a definite superiority over Bordeaux mixture in fungicidal activity. Various field tests in Illinois in 1946 have indicated that it has no notice-

able harmful effect on apples, grapes, cherries, or tomatoes. No serious injury was observed when peaches were sprayed with concentrations of $\frac{1}{4}$ lb. per 100 gal. of water or less. In greenhouse tests no injury was observed on beans when they were sprayed three times at weekly intervals

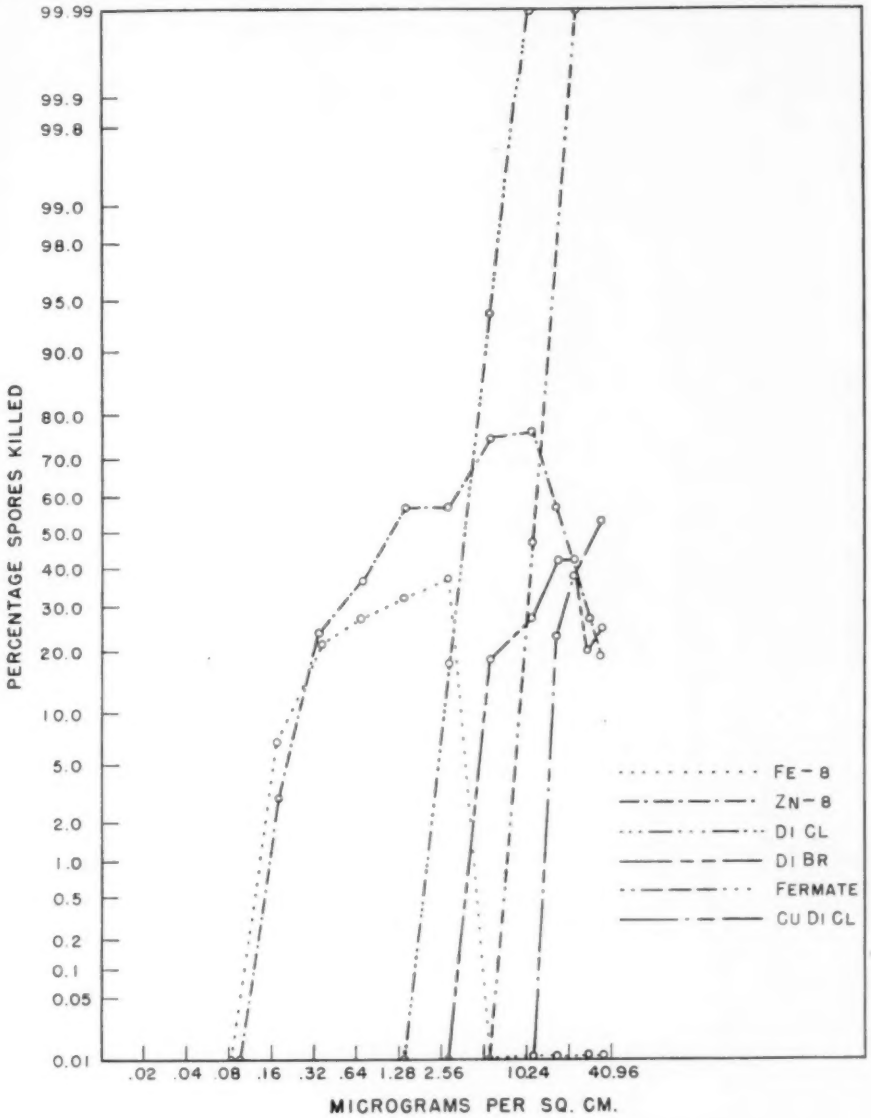


FIG. 3. Continuation of figure 2.

with concentrations as high as 4 lb. per 100 gal. Furthermore, this compound is only very slightly water soluble and will adhere to foliage for several weeks even when exposed to heavy rain. No injurious effect to man has been observed.

The pronounced variations of these compounds in their ability to affect

the fungi tested is particularly noteworthy when one considers the chemical similarity of the compounds. Zentmeyer (23 and 24) has advanced the idea that the toxic action of 8-quinolinol may be due to its precipitation of the heavy metals essential to the test organism. By adding zinc to

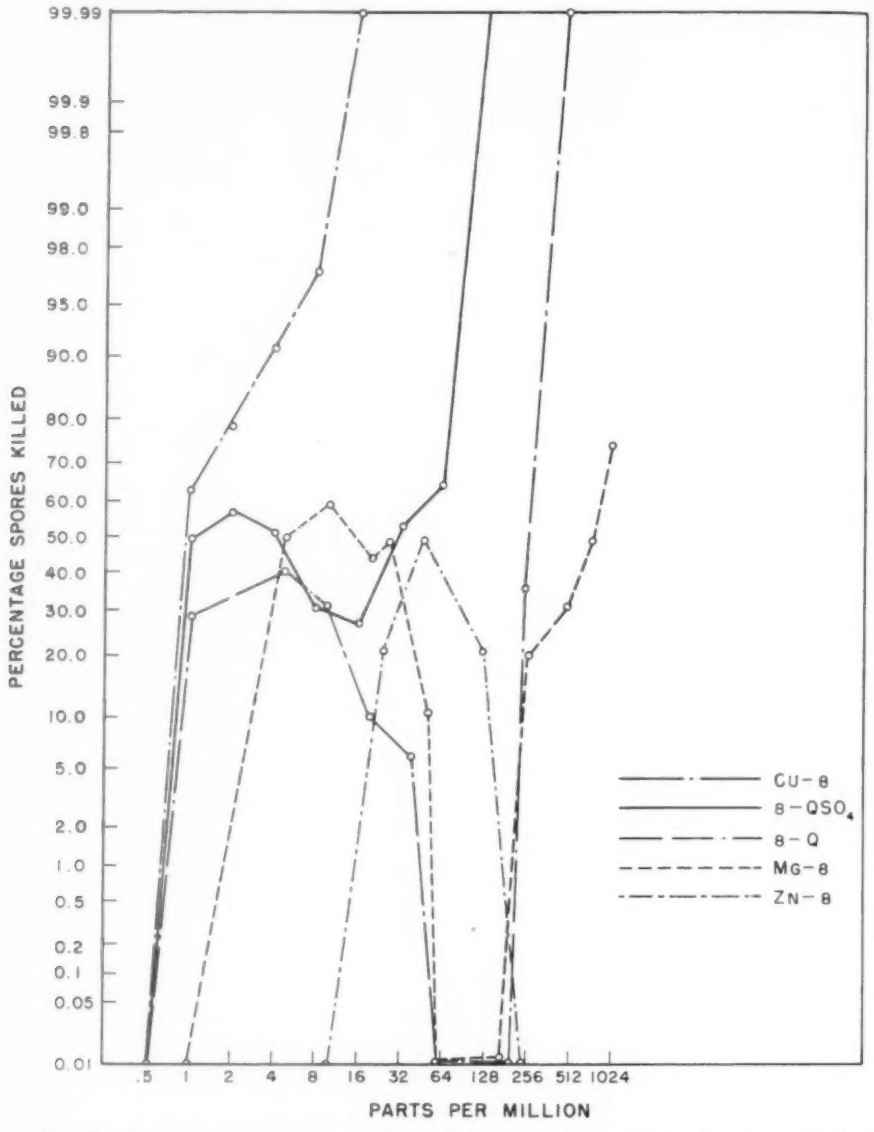


FIG. 4. Dosage-response curve of *Stemphylium sarcinaeforme* by the spore-dilution-drop method.

8-quinolinol he was able to nullify the effect of the 8-quinolinol. This theory would not explain the superiority of copper 8-quinolinolate over 8-quinolinol as observed in these experiments, since the 8-quinolinol would already be saturated with copper. Furthermore, 8-quinolinol is less efficient than the magnesium, manganese, and zinc salts in some parts of their

dosage-response curves (Fig. 4). According to Zentmeyer's theory it should be expected that 8-quinolinol would always be more efficient than either of its heavy metal salts.

For compounds showing the type of reactions reported here, it is ap-

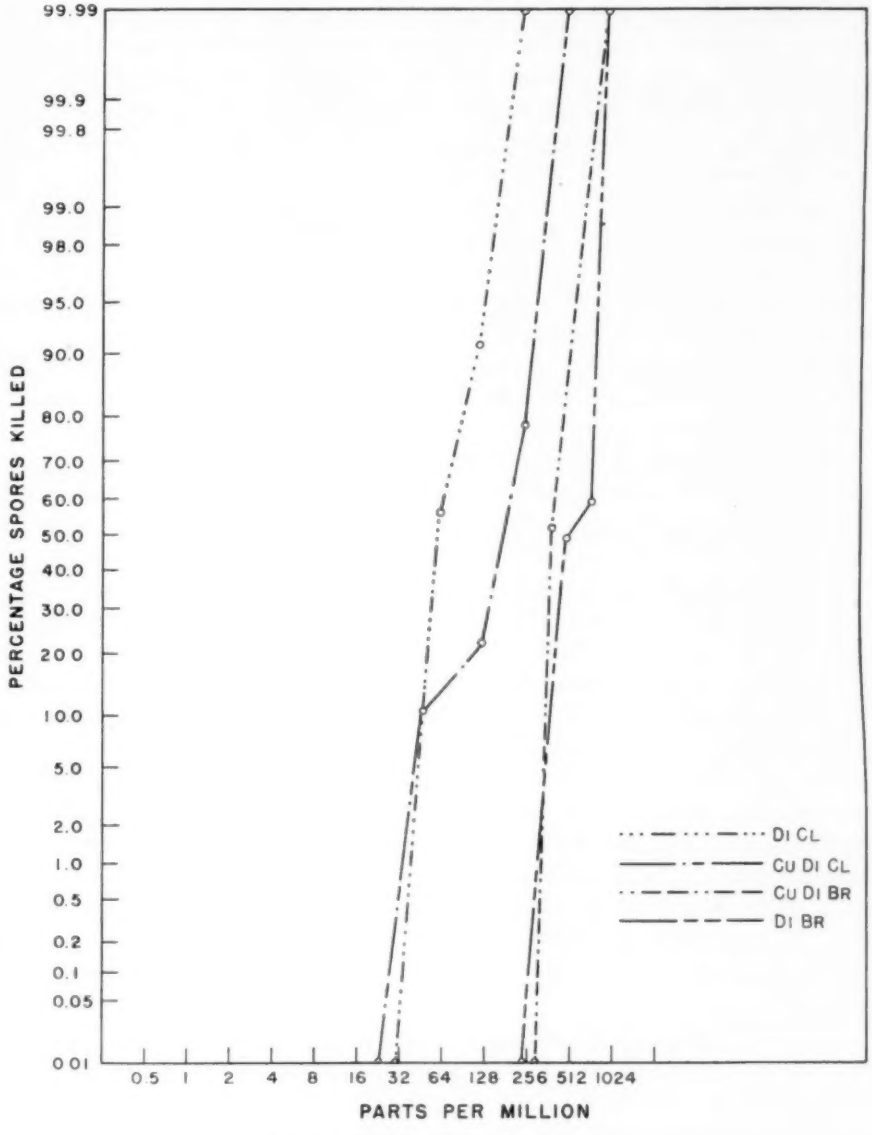


FIG. 5. Continuation of figure 4.

parent that the L.D. 50 is not a suitable index of comparison. McCallan *et al.* (15) stated that for such compounds the L.D. 95 is a much better index of comparison. If the compound shows any promise as a fungicide, it is suggested that a determination of the entire range of dosage-response and even beyond the point of L.D. 100 is desirable in order to make certain

whether there is a double effect as observed in this group of compounds. This point should not be difficult to obtain in laboratory tests with a powerful fungicide.

SUMMARY AND CONCLUSIONS

Fungicidal tests of 8-quinolinol and eleven of its derivatives by two different methods were studied. The results were tabulated and a wide range of activity for these chemically related compounds was observed. All of the metal salts of 8-quinolinol except the copper salt produced a two-peaked curve in their effect on the germination of spores of *Stemphylium sarcinaeforme*.

The substitution of chlorine or bromine atoms for hydrogen in the 5 and 7 positions was found to reduce the toxicity of 8-quinolinol. This reduction in toxicity was also observed in the copper salts of these derivatives. The substitution of nitro groups in the 5 and 7 positions eliminated the toxic action of 8-quinolinol entirely.

Copper 8-quinolinoate was found to be more effective than Bordeaux mixture in reducing the germination of spores of *Stemphylium sarcinaeforme*.

The conversion of concentrations from parts per million to molarity did not eliminate the wide variations in concentrations of compounds with different molecular weights necessary to reduce or prevent the germination of spores of *Stemphylium sarcinaeforme*.

The compounds of this group which at some point permitted no germination of spores of *Stemphylium sarcinaeforme* were found to be fungicidal rather than fungistatic.

From the results of these studies the following conclusions may be made:

1. These compounds did not produce a straight line dosage-response curve as most other compounds do.

2. The fact that not all compounds followed a straight line dosage-response curve makes the use of L.D. 50 a doubtful index for comparing one fungicide with another, unless the entire dosage-response curve from L.D. 0 to L.D. 100 has been determined.

3. The precipitation of the heavy metals by 8-quinolinol so that they are no longer available to the test organism does not explain completely the toxic action of 8-quinolinol and its derivatives tested in these experiments.

4. Substitution of chlorine and bromine atoms for hydrogen atoms in the 5 and 7 positions of 8-quinolinol did not result in an increase in the toxicity of the compound as has been reported for halogen substitutions in some phenol compounds.

5. Of the compounds tested copper 8-quinolinolate proved to be the most efficient fungicide, and it was found to have many properties desirable in a good plant protectant.

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PHYTOPATHOLOGICAL NOTES

New Hosts for Botrytis Elliptica.[1]—*Botrytis elliptica* (Berk.) Cke., the cause of a well-known leaf rot and spot of *Lilium* spp., was observed on two uncommon non-lily hosts in western Washington in 1947. Because of the potential threat to the expanding bulb and corm industry in the Pacific Northwest it is deemed important to briefly point out these cases of increase in known host range of this species. The newly noted hosts are autumn crocus (*Colchicum autumnale* L.) which was found infected near Olympia and the following varieties of *Gladiolus* spp. growing near Auburn: Rosa van Lima, Greta Garbo, Bach, Anna Mae, Cave-Cole and Jeannie.

On *Colchicum* the organism caused severe spotting of the older leaves and leaf-tip killing of the younger ones. The spots were circular to elongate, brown to yellow-brown with pale centers. Grayish-black spore masses were found on all affected parts. The numerous conidiophores were at first hyaline with pale brown walls, later becoming olivaceous-brown. They bore clusters of elliptical to elongate, hyaline, later pale brown conidia at their tips (Fig. 1). The conidia measured 17.5–35.5 × 10–14 μ (mean size 28.0 × 10.5 μ).

FIG. 1. Camera lucida drawing × 700, showing spores of *Botrytis elliptica* and apical portions of conidiophores with typical branching.

On *Gladiolus* spp. the same organism caused a very severe spotting on seedling stock, often completely killing the young leaves. The spots were numerous, small (2–5 mm. diam.), circular to slightly elongate, red to reddish-brown with slightly raised centers (Fig. 2). No fruiting structures were observed on the leaves in the field, but isolations showed the typical hyaline, elliptical conidia of *Botrytis elliptica*. The conidia measured 20–34 × 10–16 μ (mean size 25 × 11 μ). Inoculation experiments using healthy *Gladiolus* leaves produced the same symptoms described above.

[1] Published as Scientific Paper No. 762, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

The inoculated leaves were incubated in a moist chamber at 20° C. Early symptoms appeared after five days.

The isolates from these new hosts agree very closely with published descriptions of *Botrytis elliptica*. Moore[2] states that clusters of elliptical, hyaline, later sometimes pale brown conidia measuring 20–25 × 14–16.5 μ (mean 24 × 16 μ) are borne on numerous branched conidiospores, 1–3 mm. long. Westerdijk and van Beyma[3] give the spore sizes as 16–34 × 10–24 μ.—NEIL ALLAN MACLEAN, Department of Plant Pathology, State College of Washington, Pullman, Washington.

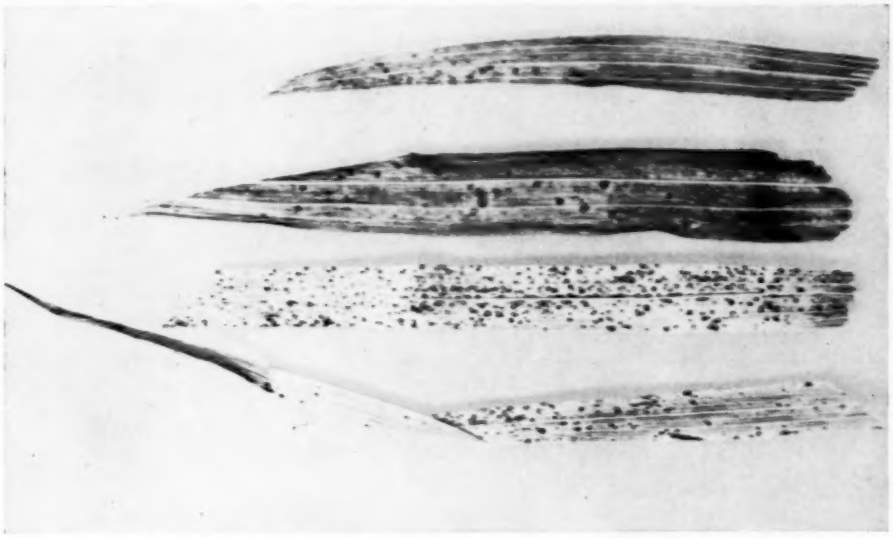

FIG. 2. Lesions produced by *Botrytis elliptica* on seedling stock of *Gladiolus* sp., variety Greta Garbo. × ½ nat. size.

Phytophthora Stem Canker of Sesame in Peru.[1]—Late in 1946 twenty-four selections of Sesame, *Sesamum indicum* L., were received at the Estacion Experimental Agricola de Tingo Maria from Centro Nacional de Agronomia, El Salvador. The first trial of these varieties was therefore during the wet season. Just prior to maturity of the fruits in late February, 1947, wilted plants were observed in the lower and poorer drained portions of the plantings and on closer examination extensive stem cankers, similar to that shown in figure 1, were found on a majority of the plants

[2] Moore, W. C. Diseases of bulbs. Ministry of Agriculture and Fisheries, Great Britain, Bul. 117: 42–46. 1939.

[3] Westerdijk, J., and F. H. van Beyma. Die Botrytis-Krankheiten der Blumenzwiebelgewachse und der Paeonie. Meded. Phytopath. Lab. Willie Comelin Scholten 12: 1–27. 1928.

[1] A contribution from the Estacion de Colonizacion en Tingo Maria, Peru, a technical agricultural service organization for the Orient of Peru, operated jointly by the Direccion de Colonizacion y Asuntos Orientales, Ministry of Agriculture of Peru and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the U. S. Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Peruvian Government.

of all varieties. These cankers were not localized in any part of the plant but were general from the collar to the tip of the growing point. Girdling infections at the collar resulted in complete wilting of the plant while infections on the upper portions usually resulted only in the loss of laterals actually involved in the infected zone. Apparently the leaves or young lateral branches served as the infection court, from which the pathogen advanced to the main stem and caused the stem cankers. These cankers were reddish brown with irregular advancing edges, and they spread vertically

FIG. 1. Phytophthora stem canker of sesame, showing wilting.

far more rapidly than horizontally. Dissection of the cankers indicated that the pathogen was primarily an invader of cambium and phloem tissue. Some infection of the fruits was also observed.

Pure culture isolations from the advancing margins of cankered areas yielded almost entirely a *Phytophthora* sp. characterized by sporangia 51.0–37.4 × 23.8–27.2 μ, averaging 39.7 × 25.5 μ, produced on branching sporangiophores, and oogonia averaging 25.5 μ with paragynous antheridia. This *Phytophthora* sp. is apparently referable to *P. cactorum* (L. and C.) Schroet.

All Sesame varieties under trial were inoculated with the *Phytophthora* by placing, in a cut extending through the bark, phloem, and cambium tissue, a small amount of mycelium from a corn meal agar culture. Ten days after inoculation extensive cankers had developed from many of the inoculations. Differences in susceptibility of the various Sesame varieties and the greater susceptibility of most of the varieties on excessively wet sites as compared to drier sites are shown in table 1.

TABLE 1.—*Length of cankers, in centimeters, ten days after inoculations on varieties of sesame, Sesamum indicum, showing differences in susceptibility of varieties on wet and dry sites*[a]

Variety[b]	TM. No.	Wet site	Dry site
Brazilian	1452	15.30[c]	2.40
Natural Indian White	1453	14.70	5.20
Indian White No. 2	1455	9.30	1.50
Indian No. 1	1456	2.30	0.00
Indian FAQ	1457	13.80	0.40
Columbian	1458	7.80	0.20
Indian Natural	1459	10.30	2.30
Brazilian White	1460	7.40	5.20
Indian No. 2	1461	15.20	3.30
Guatemala	1462	11.10	0.20
Nicaraguan	1463	12.20	1.60
Bombay White	1464	8.20	1.80
Mexican White	1466	11.20	6.50
Java Black	1467	11.70	2.10
Mexican	1468	10.90	6.20
Nicaraguan White	1469	12.60	10.60
Syrian Reddish	1470	1.80	2.10
Chinese Yellow	1471	3.60	2.90
Turquestan 2685	1472	12.80	8.10
Turquestan 2683	1474	12.30	8.20
San Salvador	1475	10.70	4.50

[a] Site differences for the last nine varieties were less pronounced than for the first twelve varieties.

[b] Three varieties, Indian White No. 1, China White, and Indian White were early maturing and could not be included in the test.

[c] Inoculations took 100 per cent, each figure represents average of 5 plants inoculated.

At present the disease, while serious, does not appear to be a limiting factor to the growing of Sesame in this region. The greater susceptibility of plants on wet sites indicates dry season crops and wet season crops on well drained sites should not be too adversely affected by the disease.

In so far as the writers have been able to determine no similar disease of Sesame, caused by a *Phytophthora*, has been reported from Peru or elsewhere; however a root rot of this species in Santo Domingo caused by an unidentified *Phytophthora* has been reported[2] and might possibly be a manifestation of the same disease.—BOWEN S. CRANDALL and JAVIER DIEGUEZ C. respectively, Pathologist, Office of Foreign Agricultural Relations, U. S. Department of Agriculture and Chief, Dept. of Plant Pathology and Entomology; and Assistant Chief, Department of Plant Pathology and Entomology, Estacion Central de Colonizacion en Tingo Maria.

[2] Ciferri, R. Phytopathological Survey of Santo Domingo, 1925–1929. Puerto Rico Dept. Agr. Jour. 14: 5–44. 1930.

A Technique for Growing Citrus Seedlings under Aseptic Conditions of Culture.—The object of devising the method described below was to grow in pure culture citrus seedlings to be used for damping-off studies with *Rhizoctonia solani* Kühn.

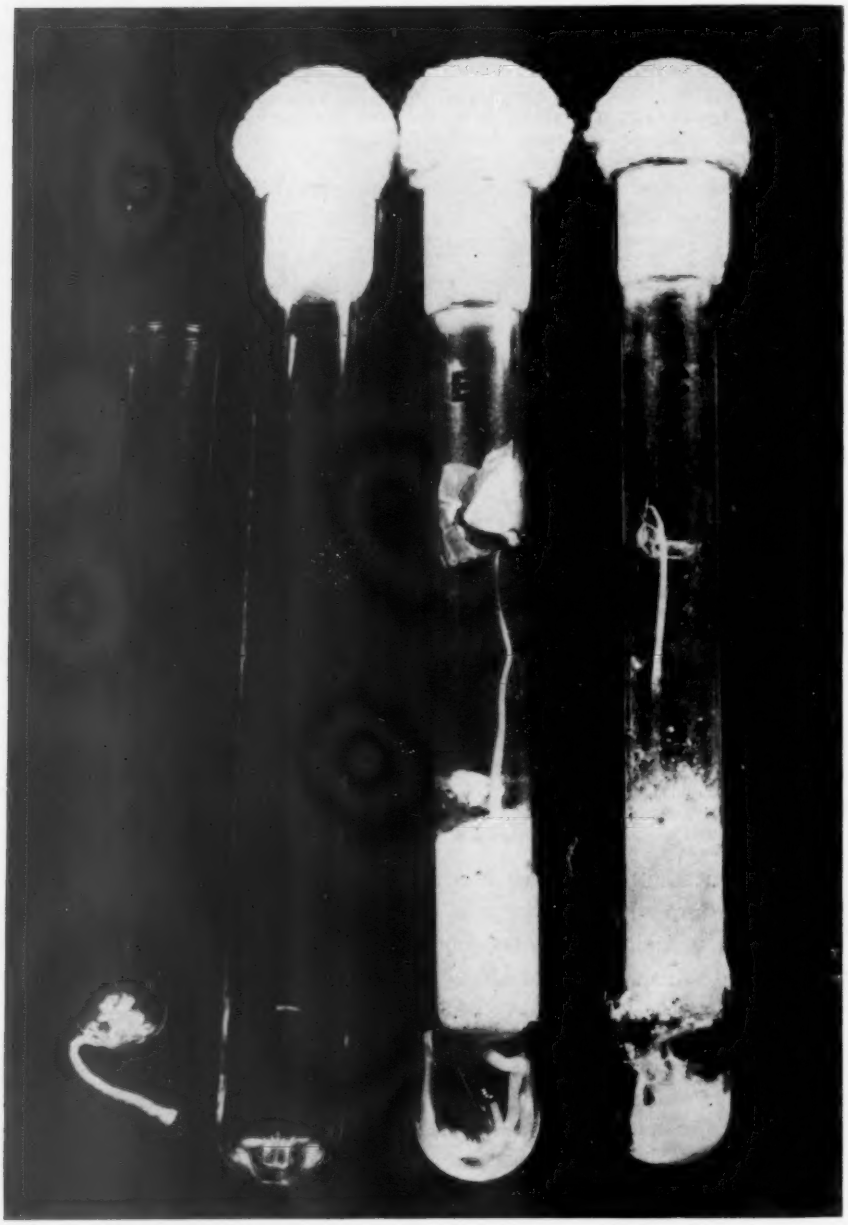

FIG. 1. Method of growing citrus seedlings under aseptic conditions of culture for inoculation with soil fungi: A. Glass test tube $6 \times \frac{3}{4}$ inches, with cotton wick passing through hole in bottom, and a larger tube of 8×1 inch dimensions containing a short glass rod. B. Healthy citrus seedling growing in quartz sand saturated with sterile nutrient solution. C. Damped-off citrus seedling after inoculation with *Rhizoctonia solani*.

With favorable temperature and soil moisture, citrus seeds usually take from 15 to 30 days to germinate. While the seeds can be germinated on sterile agar in test tubes, the procedure is less efficient than the one here described because of the following handicaps: 1. The surface of the agar in a test tube has to be broken before planting the seed; otherwise, after 3 to 4 days' incubation at 31°–33° C., it will become too hard for the young radicle to penetrate. 2. The seed does not obtain enough moisture from the agar to germinate within a normal time, thus delaying the germination unnecessarily. 3. After 15–20 days of incubation at 31°–33° C., the agar has shriveled so much that the young roots are found to hang along the walls of the test tube, where because of lack of moisture they dry and curl.

In order to overcome these handicaps and to secure seedlings under sterile conditions, agar was replaced with sand. With a blow-torch a hole about $\frac{1}{8}$ inch in diameter was made in the bottom of an ordinary glass test tube of $6 \times \frac{3}{4}$-inch dimensions. A cotton-thread wick about $1\frac{1}{2}$ inch long and $\frac{1}{8}$ inch in diameter, and with a knot in one end, was inserted through the hole so that the knot rested within the tube (Fig. 1, A). The test tube was filled with previously washed and dried fine quartz sand to a depth of $1\frac{1}{2}$ inches. The small test tube thus equipped was placed carefully into a larger glass test tube of 8×1-inch dimensions and having a one-inch piece of a glass rod and about 15 ml. of nutrient culture solution in the bottom. The larger test tube was plugged with cotton and autoclaved 20 min. at 15 lb. pressure.

After the contents of the tube are cool, one seed per test tube was planted aseptically and incubated at 31°–33° C.[1] The sand saturated with nutrient culture solution not only supplied enough moisture for germination of the seed but also fed the young roots for at least two months during which period inoculation experiments could easily be concluded (Fig. 1, B and C).

This device may also be employed for other types of seeds that require a relatively long time to germinate.

In this apparatus with the nutrient in the pH range, 4.85 to 8.85, and the incubation temperature at 25° C., a virulent strain of *Rhizoctonia solani* will damp-off sweet-orange seedlings within 3 to 5 days after inoculation.

The writer wishes to express his appreciation to Dr. L. J. Klotz for his guidance in the preparation of this note.—INAM U. KHAN, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

A Method of Testing Beans for Resistance to Bacterial Blights.—A program of breeding bush snap beans (*Phaseolus vulgaris*) with resistance to the common blight (*Xanthomonas phaseoli* [E. F. Sm.] Dowson) and the halo blight (*Pseudomonas medicaginis* var. *phaseolicola* [Burkh.] Stapp and Kotte) has been proceeding at the U. S. Regional Vegetable Breeding Laboratory since 1938. It has resulted in several new garden types, including Fullgreen No. 1 and No. 2, B1788, and B2051, now on trial with seedsmen.

[1] Fawcett, H. S. Temperature experiments in germinating orange seeds. California Citrograph **14**: 515. 1929.

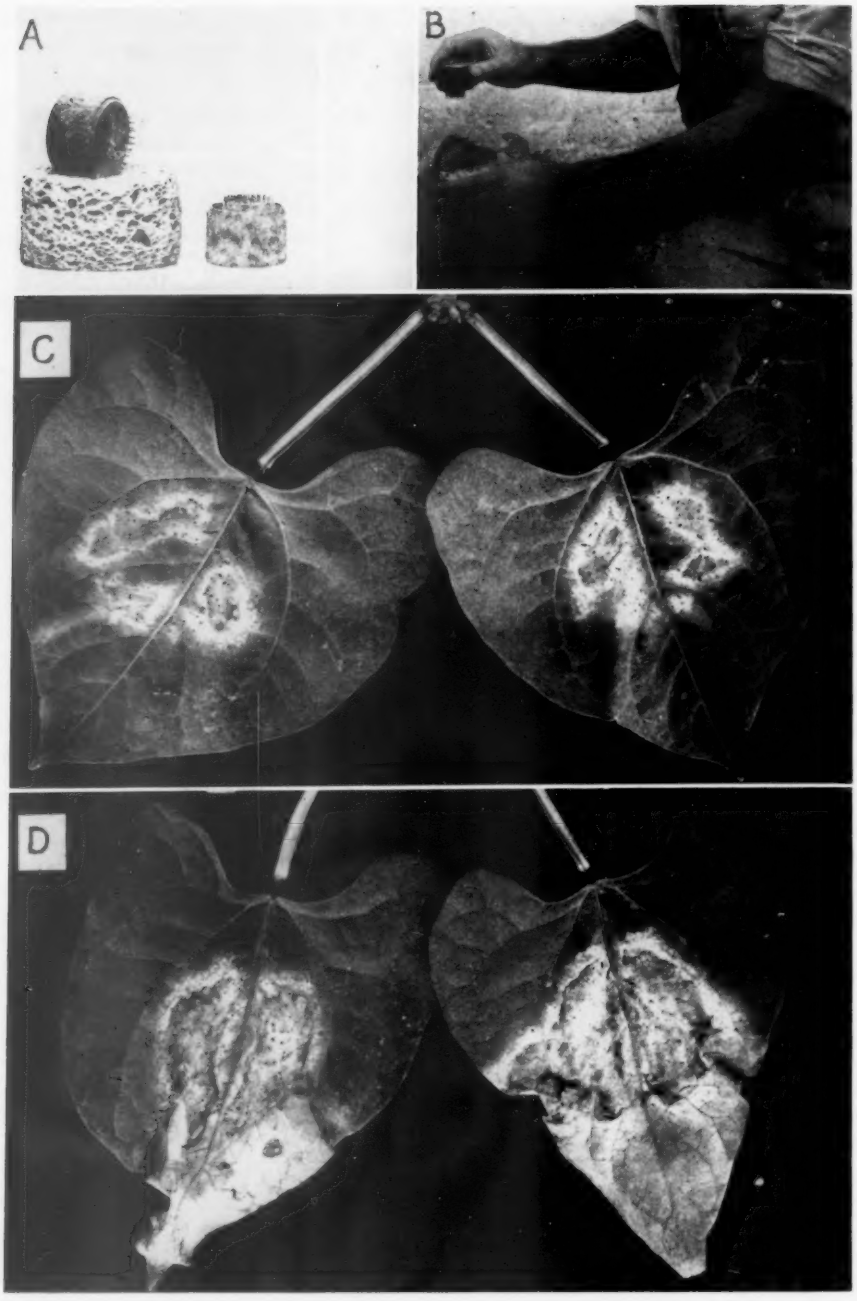

FIG. 1. Bacterial inoculation of bean leaves. A. Multiple needle inoculators and cellulose sponge. B. Inoculation of bean primary leaf. C. A resistant reaction to *Xanthomonas phaseoli* 14 days after use of the multiple needle inoculator. D. A susceptible reaction 14 days after use of the multiple needle inoculator.

During this period we have tried many different methods of inoculating bean plants to differentiate the various grades of resistance or susceptibility. These methods have included high-pressure and low-pressure sprays, seed-soaking, seed injection and stem injection with hypodermic needles, and leaf abrasion. All were discarded in 1942, and in all tests since then a new technique has been employed, which we call the "multiple needle, primary leaf inoculation."

The multiple needle inoculator was constructed with 90 No. 0 insect mounting pins with their head ends imbedded in a block of sealing wax (Fig. 1, A). The primary leaf is placed upon a rubber or cellulose sponge saturated with bacterial suspension (Fig. 1, B) and two strokes are used in passing the inoculator through the leaf into the sponge. The first stroke forces the leaf into close contact with the wet sponge and the second stroke draws additional inoculum into the leaf.

Plants are grown in pots and inoculated on about the 14th day, or when the first trifoliate leaf is beginning to open. Readings are made 12 to 14 days after inoculation, and are based on the amount of breakdown of tissues extending from the inoculated area. In order to produce a more uniform development of the primary leaves, all terminal and axillary growth is kept pruned away.

The method offers two principal advantages: (1) There are no escapes; and (2) it provides a reaction area that can be measured quantitatively (Fig. 1, C, D). Also it has emphasized two important environmental influences on bean reaction to these blights: (1)At high temperatures (above 80° F.) all varieties tend to appear susceptible; and (2) abnormally weak plants or plants attacked by root rots (*Rhizoctonia, Fusarium*) tend to have an increased susceptibility.

In a highly blight-tolerant line, a weak plant may be rather susceptible; likewise in a very susceptible line, an exceptionally vigorous plant may appear tolerant. This emphasizes in another way the importance of high-quality seed. The beneficial effects of western-grown bean seed may not be due entirely to its freedom from blight. Home-grown seed in many districts is apt to be weak, improperly matured, poorly graded, and in general will give a higher percentage of weak plants that are hyper-susceptible to blights.

The method described can be adapted to other bacterial diseases of plants. —C. F. ANDRUS, U. S. Department of Agriculture, Regional Vegetable Breeding Laboratory, Charleston, South Carolina.

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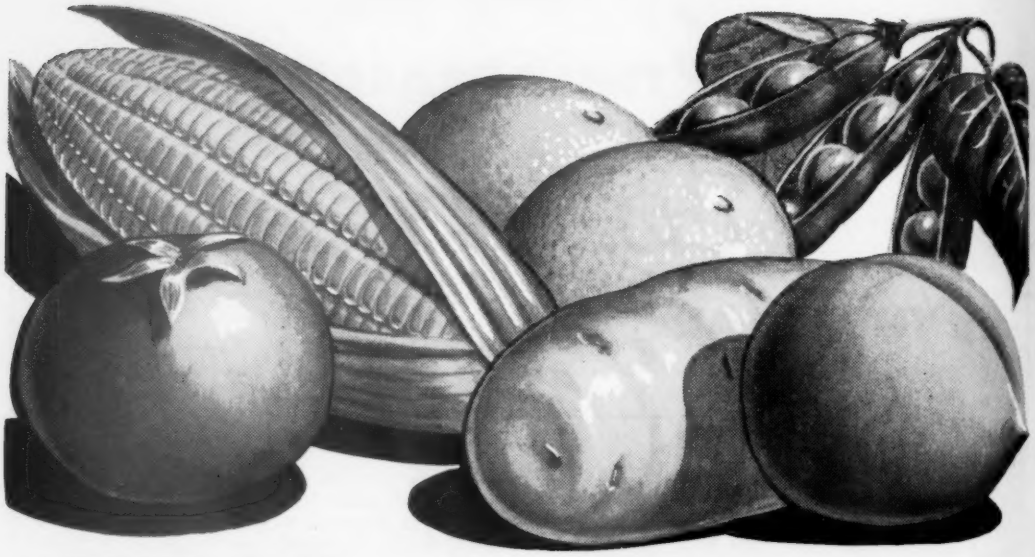

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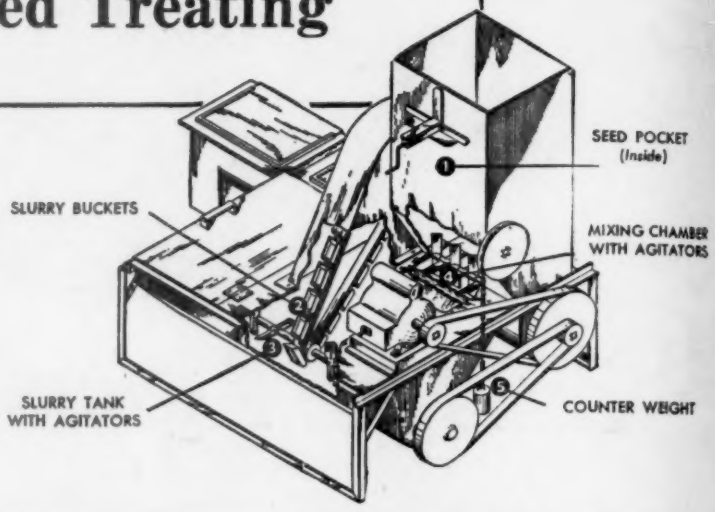

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